Investigations on Esparto Cellulose.

- by -

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I N D E X

I N D E X (cont.)

INTRODUCTION

Cellulose received its name from the fact that it is the main constituent of the cell-walls of the higher plants. In 1832 Payen (1) carried out a series of researches on very young plant formations, since great difficulty was experienced in purifying the plant skeletons of older plants from encrusting woody and fatty materials, and realised that starch was present as a reserve carbohydrate, and that there must be another polysaccharide present in the permanent structure of the cell wall. As a result of this series of investigations Payen concluded that the main skeletal tissue of all young plants consisted of one uniform carbohydrate built up of glucose units. This conclusion was naturally based on inadequate data, as really reliable methods of distinguishing between the different monosaccharides were not developed until after 1880. It was difficult to persuade the botanists of this period to believe in this one uniform carbohydrate as preparations from different plants varied in staining reactions and solubility in cuprammonium hydroxide.

As a result of extensive research it has been established that cellulose is built up of anhydro-β-glucose/

β-glucose units, linked 1:4 as in cellobiose.

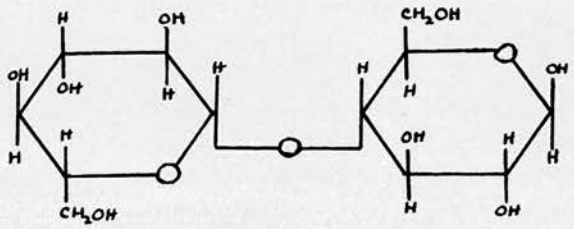


Diagram I

Cellobiose 4(β-D-glucopyranosido)-D-glucopyranose.

The fact that cellulose was built up from glucose units alone was demonstrated by Irvine and Soutar (2) who prepared the triacetate and hydrolysed it to α- and β-methyl glucosides; by Monier-Williams (3), who hydrolysed cellulose to glucose in 90.6% yield using 72% sulphuric acid; and by Irvine and Hirst (4), who improved the acetylation technique and prepared a cellulose triacetate in 99% yield. Methanolysis of this cellulose triacetate gave a crystalline mixture of α- and β-methyl glucosides, accounting for 95.5% of the cellulose used; the furfural test for pentosans was negative, and no other compounds besides the methyl glucosides were detected. This work was generally accepted as proof that pure cotton cellulose consisted entirely of glucose residues.

Each glucose residue in cellulose was considered/

considered to contain three free hydroxyl groups, from the fact that the highest acetate was found to be a triacetate (5), the highest nitrate a trinitrate(6), and the maximum possible degree of methylation found by Irvine and Hirst (7) corresponded to a trimethyl cellulose.

Further work by many investigators showed that the free hydroxyls occupied positions 2, 3 and 6, leaving positions 4 and 5 for ring formation and linkage of the units. To determine whether the glucose units possessed a 1:5 or a 1:4 ring structure, whether the units were joined 1:4 or 1:5, and whether the glucosidic bond had an α- or β-configuration, products intermediate between cellulose and glucose were investigated. Scission products like cellobiose, cellotriose, cellotetrose and cellopentose and the respective fully methylated products, were studied, and since the first three of these oligosaccharides were found to possess 1:4 β-glucosidic linkages, it seemed very probable that the glucose residues in cellulose were also linked in this way.

Although proof of the constitution of cellobiose (8, 9) provided evidence that the gluco-pyranose residues were mutually joined through glucosidic/

glucosidic linkages in 1:4 positions, this could not be taken as proof that cellulose was composed of a chain of cellobiose units since it was possible that cellobiose was a reversion product of acetolysis and was not preformed in the cellulose. Haworth, Hirst and Thomas (10) studied the graded acetolysis of trimethyl cellulose under conditions which varied from exceedingly mild to more drastic treatments. Under the milder conditions, more complex degradation products were obtained, including cellotriose and a cellodextrin, which it was considered was probably cellotetrose. Experimental evidence showed that the methylated triose was constituted on a basis of three glucopyranose units joined by β-glucosidic links, and there was no other evidence of any other type of union than of three such pyranose groups joined in the form of a chain through positions 1 and 4. The breakdown of the cellulose chain was thus shown to be progressive, from cellodextrins of unknown size, to the triose, the biose and finally to glucose itself.

Further proof of the constitution of cellobiose and cellotriose was furnished by Freudenberg and Nagai's synthesis of cellobiose (11) and the synthesis/

synthesis by these same authors of cellotriose and the methylated cellotrioside (12). Zechmeister and Tóth (13) partially hydrolysed cellulose with very concentrated hydrochloric acid and were able to isolate and identify the same intermediate products between cellulose and glucose. Freudenberg (14) regarded all these concordant and extensive researches as amounting to a chemical proof of the structure of cellulose. The presence of a simple chain structure was also deduced from a study of the rotations and kinetics of cleavage of cellulose hydrolysed by strong acids at moderate temperatures (14).

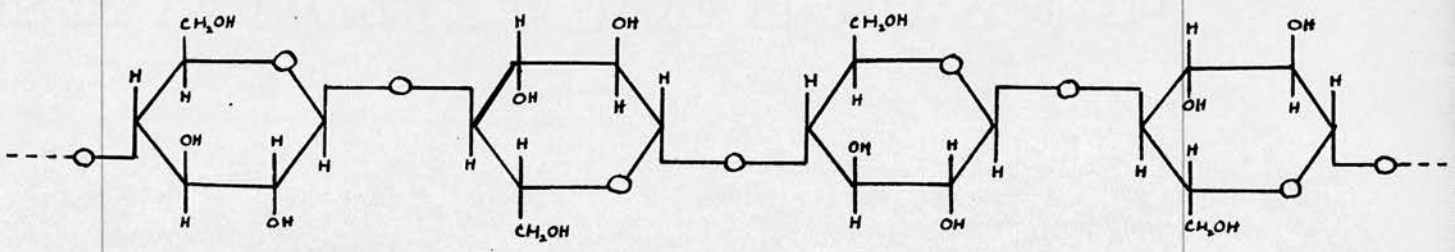


Diagram II

Cellulose.

The molecular chains present in a sample of cellulose may not necessarily be of the same length, thus an estimation of the molecular weight or degree of/

of polymerisation, of cellulose will give what may be regarded as an average value. The different ways of determining the molecular weight include end-group analyses, determination of viscosity, osmotic pressure measurements, the use of the ultracentrifuge as developed by Svedberg, and X-ray measurements. Two main methods of end-group analysis exist, depending on the fact that the molecular chain of cellulose is considered to possess two types of end-groups - a reducing group at one end of the chain and a non-reducing group at the other end. The reducing power is measured by the copper number method, or by the iodine number method of Bergman and Machemer (15), assuming that each chain molecule terminates in a reducing group and that all the reducing power is due to these terminal groups.

Haworth and Machemer (16) calculated the molecular weight of cellulose from the non-reducing end-group by acetylating cellulose to the triacetate, hydrolysing and methylating. This methylated sugar was hydrolysed, the sugars isolated and converted to methyl glucosides, from which mixture the tetramethyl glucose was isolated, and the chain length calculated from this.

Hirst and his co-workers (17) have recently developed/

developed a method which is claimed to liberate formic acid quantitatively from both terminal sugar residues of cellulose. According to these authors, a saturated aqueous solution of potassium periodate, under certain specified conditions, will not cause over-oxidation, and the formic acid liberated from the end-groups may be titrated with dilute alkali.

A disadvantage in using physical methods like viscosity and osmotic pressure methods for determining the molecular weight of cellulose, as distinct from a derivative, is the lack of solvents available for dissolving cellulose. Concentrated mineral acids attack the cellulose molecule until finally only D-glucose remains, and although cuprammonium solution is not considered to attack the cellulose, when oxygen is present, degradation takes place.

One of the most striking properties of cellulose, and of all natural substances of high molecular weight, is the high viscosity a relatively dilute solution possesses in cuprammonium solution, and since the viscosity of solutions of equal concentration has been found to be approximately proportional to the molecular weight of the solute, a method of determining molecular weights by this method has been developed, chiefly by Staudinger (18). Accurate values for the molecular weight/

weight of cellulose determined by viscosity and osmotic pressure methods depend on the extrapolation to infinite dilution from regions of concentration of 0.5% to 2% solutions - where reasonably accurate determinations can be carried out.

Values of the molecular weight of cellulose obtained by chemical, end-group, methods, indicate a chain length of from 100-200 glucose residues, while physical methods indicate the presence of about 3000 units in the chain. The higher values obtained by physical methods suggest that aggregation takes place, an assumption which is borne out to some extent by X-ray investigations of cellulose fibres.

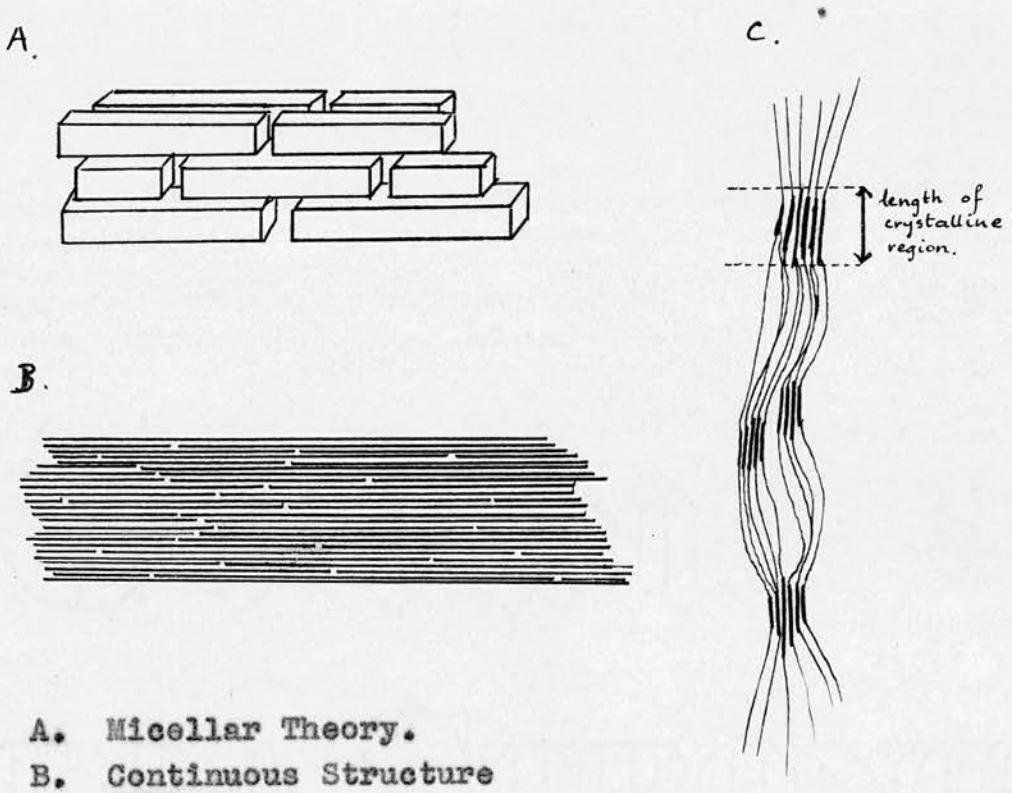
X-ray studies of the cellulose fibres have shown that the fibres possess a regular arrangement and orderly repetition, and that they are composed of crystalline aggregates and not single crystals. Meyer and Mark (19) adopted and expanded the micellar theory postulated by earlier workers, in an attempt to explain X-ray, as well as other physical and chemical data. This micellar theory postulated the presence of discrete, rod-like, sub-microscopic crystalline particles orientated with respect to the fibre axis, and separated by amorphous material which allowed the micelles/

micelles to move as a unit during intermicellar swelling and to be dispersed during the early stages of dissolution (see Diagram III (A)).

Modern theory tends rather to regard cellulose as having a continuous structure of long cellulose chains which have crystallised in such a manner that crystalline regularity is intercepted by warped or irregular regions which behave as amorphous matter towards X-rays and the penetration of swelling and dispersing agents (20, 21) (see Diagram III,(B)). A 'micellar network' theory has been advanced as a compromise between an extreme micellar and extreme continuous theory. The crystalline areas are considered not to be clearly separated by a distinct micellar surface, but rather by an area of partially parallel but disorganised chains. A netlike structure is thus postulated in which chains occur that are partly crystalline and partly amorphous (see Diagram III, (C)).

Diagram III /

Diagram III. Schematic representation of several possible crystallite structures (Mark [22]).



A. Micellar Theory.
B. Continuous Structure Theory.
C. Fringe Micellar Theory.

If, as has been suggested, the dimensions of the crystallite of cellulose are of the order of 500-600 Å units by 50 Å units, about 60 cellulose chains each with a length of 100-120 glucose units could be packed into each crystallite. A micellar length of 100-120 units is similar to the minimum molecular size of 100-200 units deduced by chemical means by Haworth (16).

Further/

Further work on the constitution of cellulose was carried out by Haworth and his co-workers (23,16), by the methylation of cotton cellulose in an atmosphere of nitrogen. After this methylation of cellulose in nitrogen, no end-groups were recognisable, and although methylation first in nitrogen and then in air brought about diminution of molecular size, presumably by diminution of chain length, no end-groups became exposed after this breakdown, or dis-aggregation. The end-groups which became exposed when the chains broke must have become involved in some form of recombination, so that none remained. End-groups remained exposed when cellulose was methylated in air, indicating that the presence of air appeared to act to some extent as an inhibiting factor towards the linking up of the ends of the broken chains.

From the experimental evidence, Haworth (24) concluded that the two ends of a short chain of cellulose must be in a suitable position to join up with the two ends of a similar chain, and that this reaction could not occur at random, but 'must constitute the major effect represented by a yield of 80-90% when the methylation is conducted in nitrogen'. The necessary/

necessary conditions are not likely to exist if the short chains of cellulose are capable of aligning themselves at random, thus Haworth was led to believe that at least two short chains were held together by intermediate bonds at various points along their lengths. The nature of these 'cross-linkages' or valency bonds was not determined but their presence would ensure the relative positions of the two chains to be such as to enable the closure of the ends of the chains (See Diagram IV.).

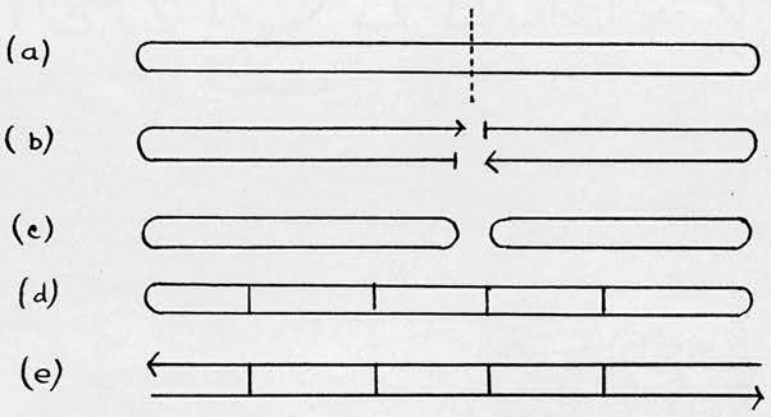


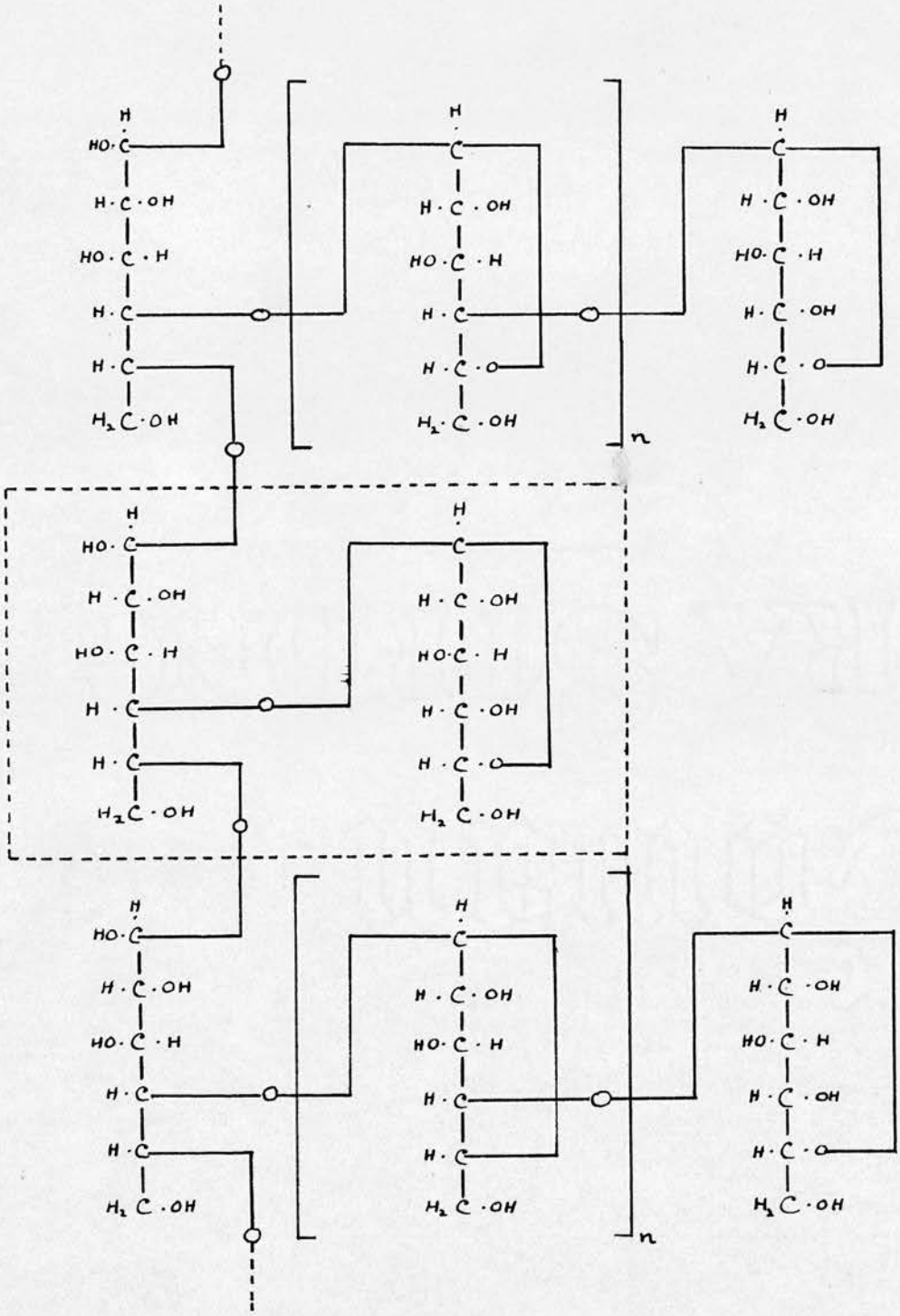
Diagram IV[25]

(a) Methylated cellulose represented as a large loop.
(b) Two open-chain structures produced by scission of loop (a).
(c) Exposed ends of both fragments unite together to form smaller loops if methylation carried out in absence of air.
(d) Alignment maintained by bonds which link across the loop.
(e) Two chains held together by intermediate bonds along their length. Ring closure of such a pair would give the looped structure (a).

In a recent publication, Pacsu (26), from the results of acid-catalysed hydrolysis of cellulose and from certain data in the literature, concluded that native cellulose possessed an infinitely high molecular weight, and that it was degraded to a degree of polymerisation of about 3000 units as a result of the procedure usually adopted. Further degradation to a degree of polymerisation of 128 or 256, resulting in complete loss of tensile strength of the fibre, was caused by mild initial acidic degradation. Pacsu also postulated the presence of about 0.3% covalent bonds which were equally spaced, acid sensitive and entirely different from the regular 1:4-glucosidic bonds, cleavage of which brings about random hydrolysis in strongly acid media (See Diagram V).

Diagram V /

Diagram V. Pacsu's Formula for Cellulose.



These covalent bonds, which are broken in the mild, initial acidic degradation, were supposed to be either acetal or hemiacetal bonds which originated from the open chain reducing units of the 'primary chain molecules'. This principle of periodicity finds support in the work of Schulz and Husemann(27) who considered that native cellulose consisted of a chain of 3×2^{10}, i.e. 3072 anhydroglucose units linked by 1:4-β-glucosidic bonds, and 5 equally spaced glucuronic acid residues, which might have become xylopyranoside units by loss of carbon dioxide molecules from their carboxyl units. Schulz and Husemann considered that the 1:4-xylopyranosidic linkages would hydrolyse about one thousand times faster than the normal glucopyranose bonds, with the result that in acid media the cellulose molecule would be rapidly degraded to six chain molecules, each of which consisted of 2^9, i.e. 512 anhydro-glucose units.

In a recent publication(41) Banderet and Ranby claim that their work on the treatment of cellulose with sodium hydroxide solution in the absence of oxygen confirms the conclusions of Staudinger (42) and Schulz and Husemann (42) that most of the glucose units/

units of the cellulose chain are united by β-glucosidic linkings, but every so far in the chain there is another type of linkage, possibly of an ester nature.

Confusion has been caused in the past by the fact that the term 'cellulose' has been applied to the polysaccharide which is hydrolysed to glucose alone, and also to those polysaccharides which form the structural constituent of the cell-walls of plants, and which yield other sugars, as well as glucose, on hydrolysis. The pure glucose polysaccharide $(C_6H_{10}O_5)_n$ only occurs alone, or at least in a relatively pure form, in the seed hairs of the cotton plant Gossypium, and to this polysaccharide has been applied the term 'true' cellulose. The other celluloses, which are more easily hydrolysed and yield other sugars in addition to glucose on hydrolysis, have been found to consist of 'true' cellulose associated with one or more associated polysaccharides. These associated polysaccharides, which have been termed 'hemicelluloses' by Schulze (28), are difficult to separate from the 'true' cellulose and are more resistant to extraction and hydrolysis when associated with cellulose than when alone. The hemicelluloses comprise both the 'polyuronide hemicelluloses' and the 'cellulosans', the latter originally/

originally forming part of the cellulosic fabric itself, and existing only associated with the true cellulose, being oriented in the micellar structure. These cellulosans may be either pentosans or hexosans, the main pentosans found being xylan, araban and the methyl pentosans, while the more common hexosans are galactan and mannan. These aggregates of 'true' cellulose and hemicellulose have been termed 'holo-celluloses.

The cellulosan most commonly found associated with cellulose is xylan, which has been shown to be a short-chain polysaccharide consisting mainly of xylose units. On hydrolysing xylan extracted from Oran esparto, Hampton, Haworth and Hirst(29) obtained xylose in 93% yield, and concluded that xylan was composed entirely of xylose units. The hydroxyl groups occupying positions 2 and 3 in the xylose units were found to be free, showing that positions 4 and 5 were concerned with ring formation and the linking of units. Haworth and Percival (30) showed that position 4 was the linkage of one xylose unit with position 1 of an adjacent xylose unit, that position 5 was the point of junction of the xylose ring, and concluded that xylan was composed of a chain of xylopyranose units joined 1:4- as in Diagram VI.

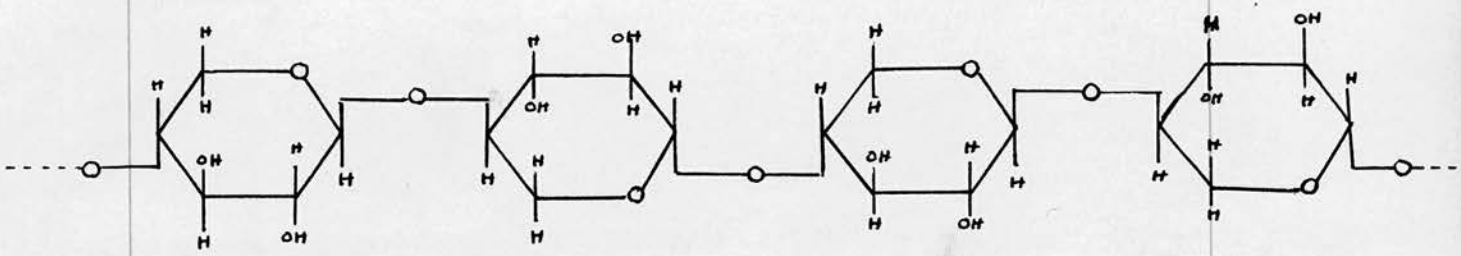


Diagram VI. Suggested Formula for Xylan - a chain of xylopyranose.units.

Continuing investigations on the constitution of xylan, Haworth, Hirst and Oliver (31) came to the conclusion that xylan prepared from different sources always contained a fixed proportion of L-arabinose, in the furanose form, as well as the xylopyranose units, and that the simplest interpretation of the experimental evidence was that xylan consisted of 18-20 xylopyranose units terminated at one end by an arabofuranose unit. Confirmation of this structure of xylan was obtained in 1937 when Bywater, Haworth, Hirst and Peat (32) removed the terminal arabofuranose unit by means of aqueous oxalic acid, leaving a new xylopyranose end-group, which was evaluated as trimethyl xylopyranoside. This excluded the possibility that the xylan structure consisted/

consisted of a continuous loop of xylose units with side chains of L-arabofuranose. Some 2-monomethyl xylose was always found to be present after methylation and hydrolysis, which suggested that there was some kind of link between the potential reducing group of one chain and position 3 of a xylose residue in an adjacent chain, but the nature of the linkage remained a matter for speculation.

Husemann (33) examined xylans from various sources and determined the molecular weight by the osmotic pressure method and found that the chain length varied from 113 to 150. Since the xylans were found to be more soluble than cellulose, although the viscosity constants were similar, it was concluded that a regular series of very short chains was indicated.

The presence of a small amount of uronic acid residues in xylan has been postulated by Haworth(24), E. Schmidt(34), and Voss, Bauer and Pfirschke (35). The experimental work of Voss, Bauer and Pfirschke bore out Schmidt's opinion that a part of the carboxyl groups did not exist free, but was originally fixed in some ester-or lactone-bond. These authors disagreed with Schmidt, however, in not considering that the carboxyl groups were primarily responsible for the cross linking of chains, since their 'easily soluble' xylan/

xylan was found to contain more carboxyl groups than 'slightly soluble' xylan, and should therefore have been more capable of linkage with other chains and of forming stoichiometric compounds.

Although there is a vast collection of literature on the subject of cellulose, and although a considerable amount of work has been done on the cellulosans which accompany cellulose in nature, the celluloses when freed from their associated cellulosans have not been the object of much research. The holocellulose extracted from esparto grass has been found to have a xylan content varying from 20-30%, depending on the conditions of extraction. When the 'true' cellulose has been freed from its associated xylan it has been concluded to be identical with cotton cellulose, although there is as yet insufficient data for this conclusion to be justified.

The first real study of esparto holocellulose was made in 1918 by Cross and Bevan (36), who found that the divergences from cotton cellulose were that esparto holocellulose yielded a high proportion of furfural on distilling with acid, and that the aniline reaction was sufficiently constant to act as a measure of the proportion of esparto holocellulose present in a mixture of cotton and wood celluloses. Esparto holocellulose was classed with the cereal celluloses/

celluloses, as they presented the same specific characteristics, but the cereal celluloses were considered to be complex mixtures, while esparto holocellulose - as used in paper-making - appeared to behave as substantially homogeneous. A high proportion of furfural was obtained with acids, but it was considered doubtful whether these so-called 'furfuroids' were pentosans, since various treatments caused a change in the 'furfuroid' groups, a change which was indicated by a fall in the yield of furfural from 12.5% to 7.5% or 8.5%.

The subject was left in this unsatisfactory state until a few years later, Irvine and Hirst(37), examining the holocellulose obtained from esparto grass, found that approximately half the weight of the dried esparto grass could be extracted by digesting the grass with sodium hydroxide solution under pressure. The residue - a valuable paper-making material - acted in many ways as a homogeneous substance, and differed from cotton cellulose in having a 'pentosan' content, detected by its yielding furfural on distilling with 12% hydrochloric acid. The pentosan content was found to vary with the conditions of extraction, but in general about 20% was present, and could be eliminated only by prolonged extraction/

extraction with boiling sodium hydroxide solution. Taking these facts into consideration, Irvine and Hirst formed the opinion that the esparto holocellulose might consist of a mixture of polysaccharides derived respectively from hexose and pentose units, or a mixed polysaccharide in which hexose and pentose units were condensed together.

Irvine and Hirst worked with a holocellulose from esparto grass containing 18.5% pentosan and obtained evidence as to the constitution of the two components by converting the pentosan-free cellulose into cellobiose and by hydrolysing the pentosan to xylose and then converting it into trimethyl β-methyl xyloside. The holocellulose itself was then acetylated and yielded a product which contained cellulose triacetate and xylan diacetate, the latter being converted into a mixture of α- and β-methyl xylosides by heating with methanolic hydrogen chloride. It was concluded that esparto holocellulose was a mixture of cellulose and xylan and not a chemical individual, since it was not considered likely that the action of the alkali would be to detach and dissolve combined pentose units in a polysaccharide and to leave the hexose units unattacked. This conclusion was supported by the fact that on the acetolysis of esparto holocellulose under/

under varied conditions, no trace of a disaccharide containing xylose was detected. Although these experiments did not show definitely whether the cellulose and xylan were mechanically mixed, or if they formed a solid solution, the latter view was favoured on the evidence of the microscopic examination of esparto fibre and the acetate to which it gives rise.

The exact relationship between cellulose and its associated cellulosans has never been elucidated completely, although various theories have been advanced, of which the solid solution theory of Irvine and Hirst is an example. The fact that associated cellulosans showed considerably more resistance to hydrolysis and extraction when associated with cellulose than when alone led Norman (38) to conclude that the form of association with the cellulose conferred this peculiar resistance on the cellulosan, or at least, on part of it. Another suggestion by Norman (38) was that there might be chains composed of 20 xylose units, and 150 glucose units, resulting in a chain length indistinguishable from that of a cellulose chain composed of between 100-200 glucose units. A few of these chains might be the explanation of the fact that/

that some of the xylan is more resistant than the rest to extraction from esparto holocellulose with sodium hydroxide solution. Against this view is the evidence of Irvine and Hirst (37), who found no trace of a disaccharide containing both xylose and glucose.

Another theory suggested that the association between cellulose and cellulosan was a special case of mixed crystallisation, a theory which was supported by the fact that removal of xylan from holocellulose fibres made no fundamental difference to the X-ray pattern. Astbury, Norman and Preston (39) found that the progressive removal of xylan from fibres of high xylan content merely made their X-ray photographs like those of low xylan content, that is, merely showing a more perfect state of crystallisation.

A later opinion of Norman's (40) was that there was no real line of separation between cellulose and cellulosan, and the distinction which was made on the basis of insolubility in dilute alkali and susceptibility or resistance to dilute acid was merely arbitrary. The cellulosans which accompanied cellulose in the structural holocelluloses of plants were considered to be relatively short chain compounts in which the xylose, mannose, etc. units would occupy, longitudinally, the same space as the glucose units in cellulose /

cellulose, since they are present also in the pyranose form, and these short-chain compounds would be retained by the same lateral forces which exist between adjacent cellulose chains. The xylan chains would not possess the projecting carbinol group on carbon atom 6, but this lack did not seem to affect the extraction of xylan, since xylan was no easier to remove from softwood holocellulose than mannan.

Voss, Bauer and Pfirschke (35) studied the removal of xylan from fruit stones, and were of the opinion that two varieties of xylan existed - 'sparingly soluble xylan' and 'easily soluble xylan'. On removing the 'easily soluble xylan', the residual holocellulose had the ratio cellulose to xylan of 3:1 or 2:1, which was explained by the assumption that the chains of xylan were arranged as a layer round a bundle of cellulose fibres. These coefficients, cellulose to xylan 3:1 and 2:1, were regarded as being the ratio of the area of an inner circle to that of an outer circle, from which it was assumed that the anhydrides of glucose and xylose occupied the same volume, although the reasons for this assumption are obscure.

The 'easily soluble xylan' was regarded as intermicellar material, mainly because of the variability/

variability in its composition. These authors did not consider that linkages between chains by ester linkages could give rise to compounds, and furthermore, were of the opinion that no compound existed as such, any more than a compound of sodium chloride exists in the rock salt lattice.

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PART I.

INTRODUCTION.

The isolation of cellulose from its natural sources presents several difficulties which prevent the encrusting materials from being removed in one single process. The methods employed must be severe enough to remove these encrusting materials, but sufficiently mild to avoid degrading the cellulose, the properties of which are easily modified by oxidation, acids and heat. Cross and Bevan's (1) original method of extraction was alternately to expose the material to chlorine - either gaseous or in solution - and to extract with hot sodium sulphite solution. The lignin was supposed to act with the halogen by substitution and partial oxidation, giving rise to 'lignone chloride'. The hot sulphite solution proved to be a solvent for this 'lignone chloride', and also for the encrusting hemicelluloses. To obtain a pulp suitable for use in the paper-making industry, the method of isolation adopted in the case of esparto grass is to extract the grass with caustic soda solution under pressure and to bleach the resulting pulp with a solution of bleaching powder. Any degradation which results from the varied conditions of extraction - pressure and time of/

of extraction and strength of alkali - and of bleaching - time and temperature of bleaching and strength of bleaching solution - can be observed by changes in the fluidity of the cellulose or pulp, as determined by the method of Clibbens and Geake (2) and Clibbens and Little (3).

This industrial extraction from esparto grass gives rise to an esparto cellulose containing approximately 17% pentosan, which can be extracted by prolonged treatment with alkali, or by a stronger solution of caustic soda. Various methods have been employed to extract the pentosan - the xylan - from esparto cellulose, in a pure form. Irvine and Hirst (4) extracted 'boiled esparto grass' with 6% aqueous alkali under 45 lbs. steam pressure and removed the colouring matter and residual lignin from the resulting pulp with moist chlorine. Xylan was extracted from this esparto cellulose by boiling gently 100 parts with 120 parts of caustic soda in 1000 parts of water, with gentle stirring for 10-12 hours. All the pentosan was removed after a second similar treatment and the dark brown filtrate cooled to 50°C. and the xylan precipitated by the addition of an equal bulk of 90% alcohol. After standing for 12 hours the supernatant liquor was decanted off and the crude xylan/

xylan, while moist, triturated with aqueous acetic acid to remove alkali, washed with water, alcohol and ether in quick succession and dried slowly at a low temperature. The xylan prepared in this way had an ash content of 3% to 4% and a pentosan content of 91%.

Hess and Lüdtke (5) extracted pine-sulphite pulp with 2% caustic soda solution for 14 hours and the xylan was precipitated from the alkaline filtrate by methyl alcohol. This crude xylan was purified by dissolving in cuprammonium solution and re-precipitating with acetic acid and methyl alcohol. This treatment was repeated several times and finally the xylan dissolved in normal caustic soda solution and precipitated with 50% aqueous acetic acid. The xylan obtained by this method showed a pentosan content of 93%, but had a high ash-content which was not removed completely even after continued washing with dilute acid.

Hampton, Haworth and Hirst (6) then improved the method adopted by Irvine and Hirst a few years previously (4) and removed all lignin from the wax-free esparto grass and bleached the resulting pulp, all under the mildest conditions possible. The resulting esparto cellulose was extracted with 12% aqueous caustic soda for 12 hours, the filtrate cooled to 35°C. and the xylan precipitated by the addition of an equal volume of methylated spirits, added cautiously/

cautiously and with continuous stirring. The xylan was filtered off and treated with a mixture of equal volumes of glacial acetic acid and alcohol, followed by water, alcohol and ether, and dried in a vacuum desiccator at room temperature. Prepared in this manner, the xylan was usually colourless, but occasionally was yellowish and was purified by dissolving in 12% caustic soda solution and re-precipitating as before.

A cellulose containing xylan was extracted from beechwood by Voss, Bauer and Pfirschke (7) by treating the beechwood with chlorine dioxide and purifying the pure white product obtained with 0.2% caustic soda solution. One portion of xylan, which these authors termed 'easily soluble xylan', was obtained from this extract with 0.2% caustic soda solution by carefully neutralising with hydrochloric acid and concentrating in vacuo to about 5% of the original volume. This concentrated solution was electrodialysed and treated with an equal volume of alcohol, the precipitate filtered off after 24 hours and extracted with alcohol and ether, and dried in vacuo. The other portion of xylan, termed 'slightly soluble xylan' was extracted from the cellulose purified with 0.2% caustic soda solution, with 5% caustic soda solution containing 3% sodium chloride, and the extract/

extract neutralised with 2% hydrochloric acid and concentrated *in vacuo* at 40°C. A paste of xylan and sodium chloride was obtained, which was dialysed for 3 days, the solution concentrated *in vacuo* and the xylan precipitated by adding an equal volume of alcohol and dried with alcohol and ether. This xylan had a high ash-content (5-8%), which was only removed by prolonged dialysis against distilled water or by electrodialysis for 24 hours.

Jayme and Sätre (8) extracted xylan from bleached straw cellulose with caustic soda solution, the concentration of both the straw cellulose and the caustic soda being 5% by weight. The xylan was precipitated from the alkaline solution by two methods - by the addition of Fehling's solution and by alcohol containing glacial acetic acid. In the former method, Fehling's solution was added to the alkaline filtrate, with stirring, and the resulting blue, gelatinous precipitate twice ground up with alcohol, then suspended in alcohol and the copper complex destroyed by introducing dry hydrogen chloride, with stirring, until the precipitate was colourless. The xylan so obtained was then washed with alcohol and ether and dried. In the second method adopted by these authors, the alkaline filtrate was poured into alcohol containing glacial acetic acid - 20 ml. glacial acetic acid for/

for every 100 ml. alcohol - and allowed to stand overnight. The xylan was filtered off and stirred up with alcohol four times, then with ether, and dried.

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PART I - EXPERIMENTAL.

Extraction of Esparto Cellulose from Tunisian Esparto Grass.

The conditions under which the cellulose was extracted from esparto grass corresponded as far as possible to the conditions under which paper-makers' pulp is extracted on the industrial scale. The esparto grass was first cut into suitably short lengths and 100 g. extracted with sodium hydroxide solution which was made up by dissolving sodium hydroxide (16.7 g.) in water (300 c.c.). It was found advisable to boil the grass and sodium hydroxide solution for a few minutes before extraction, until the grass had softened sufficiently to allow it to be all pressed down into the liquid and kept in place with a perforated lead disc. The extraction was then carried out for 4 hrs. in a 'Pentecon' pressure heater at 40 lb./sq. inch pressure, when all the lignin and some of the encrusting hemicelluloses were removed. The resulting pulp was filtered off from the liquor on a 15 cm. Buchner funnel, the pulp forming its own filter pad, and bleached for 4 hours with a solution of bleaching powder (water (85 c.c.), bleaching/

bleaching powder (4.7 g.)), washed with water until free from all traces of the bleaching solution, then with alcohol and ether and allowed to dry in the air.

I. Effect of Conditions of Extraction on Fluidity.

The conditions under which the pulp was bleached were modified in various ways, in order to ascertain whether the bleaching process was liable to degrade the pulp to any serious extent. Possible degradation in the pulp was indicated by determining the fluidity of the pulps which had been bleached under different conditions. The conditions of extraction with sodium hydroxide solution were then varied to see how the fluidity was affected by various conditions.

All fluidity determinations were carried out using the X-type of viscometer, as described by Clibbens and Geake (1) and Clibbens and Little (2). This is a capillary instrument specially designed to simplify the measurement of fluidity for a system which is very sensitive to the presence of atmospheric oxygen.

The viscometer is of certain specified dimensions and consists essentially of a long wide glass tube forming the upper portion, which also acts as a solution vessel for the cellulose; the lower portion of the instrument consists of a short glass capillary /

capillary, which discharges the solution into the air. The correct weight of cellulose necessary to give a 0.5% solution is dissolved in the cuprammonium solution by attaching the viscometer to the spokes of a bicycle wheel which revolves at a maximum speed of 4 revolutions per minute. A specified volume of mercury (0.7cc) is introduced into the instrument to act as a stirrer during dis-solution by falling from end to end of the liquid column as the viscometer revolves. The agitation produced in this way by overnight running is sufficient to ensure complete and homogeneous solution of the cellulose. This homogeneous solution is then allowed to flow freely through the lower capillary of the viscometer and the time necessary for the meniscus to travel between the upper and lower timing marks noted. All determinations were carried out in a thermostat at 20°C. Each viscometer supplied by the British Cotton Industry Research Association, Shirley Institute, Didsbury, Manchester has its own calibration data, from which, together with the measured time of flow of the cellulose solution, the fluidity can be calculated.

As a standard the fluidity of "standard cellulose" was determined. This "standard cellulose" was supplied by the Shirley Institute and was cotton cellulose extracted under the mildest conditions possible, and found to have a fluidity of 2.46 c.g.s. units /

units. This represented the fluidity of an almost undegraded cellulose, while although the fluidity of the solvent itself was 72 c.g.s. units, a fluidity of over 40 c.g.s. units indicated that the cellulose was almost completely degraded.

Among the calibration data supplied with each viscometer was the weight of cellulose necessary to give a 0.5% colution, assuming the cellulose had a moisture content of 6%. The moisture contents of the air-dried pulpswere determined by heating under reduced pressure at 60°C over P_2O_5. The moisture contents were found to vary from 6% to 13%, so the weights of pulp with a moisture content of 6% were calculated.

(1) Variation in Fluidity with Time of Bleaching.

Esparto grass (100g.) was digested for 4 hours with water (300cc.) containing sodium hydroxide (16.7g.) under 40lbs/sq.inch pressure. The resulting pulp was washed with water until free from alkali, and equal portions bleached for varying periods with equal amounts of bleaching solution. The bleaching solution was made up from water (85c.c.) and bleaching powder (4.7g.) and the insoluble portion filtered off. The proportion of bleaching solution to grass used was 85 c.c. for every 100g. of the grass originally digested. The bleached pulps thus obtained were washed under

running /

running water until free from any trace of the bleaching solution, then with alcohol and ether and dried in the air, and the fluidities determined. After bleaching for 1 hour the fluidity was found to be 6.18c.g.s. units and rose to 6.46c.g.s. units after 2 hours, to

7.37 after 5 hours, to

7.97 after 24 hours, and to

8.72 c.g.s. units after 48 hours.

Very little degradation would appear to have taken place in the pulp, even after being in contact with the bleaching solution for 48 hours.

(2) Variation in Fluidity with Temperature of Bleaching.

Esparto pulp was extracted from esparto grass as in (1) above, by digestion with alkali. Equal portions of this pulp were then bleached for 4 hours with equal volumes of the same bleaching solution, as in (1), at different temperatures. The resulting bleached pulps were washed until free from all traces of the bleaching solution, washed with alcohol and ether and dried in the air and the fluidities determined. A fluidity of 7.1c.g.s. units was obtained for the pulp bleached at $0^{o}C$ and this rose to

8.0 at $10.5^{o}C$,

8.0 at $20^{o}C$

8.4 at $30^{o}C$ and

8.5 at $45^{o}C$.

No /

No significant rise in fluidity, and hence no significant degradation of the pulp was encountered up to a temperature of 45°C.

(3) Variation in Fluidity with Strength of Bleaching Solution and Proportion of Bleaching Solution to Pulp.

Esparto grass (100g.) was digested with sodium hydroxide solution as in (1) and washed free of alkali. Equal portions of this pulp were then bleached using different concentrations of the bleaching solution and different proportions of the volume of the bleaching solution to the same weight of pulp. The usual strength of bleaching solution was used first-water (85c.c.) and bleaching powder (4.7g.), 85c.c. of the solution being used for every 100g. of the grass originally digested. Next, five times this volume of the same bleaching solution was used for the same weight of grass. A bleaching solution of double this strength was then used - water (85cc.) and bleaching powder (9.4g.), using the 85 c.c. per 100 g., followed by five times this volume of the stronger bleaching solution for the same weight of grass. The fluidities obtained can be seen from Table I.

Table I

Strength of Bleaching Solution Used. Volumes given are for 100 g. of the Grass	Temperature °C	Fluidity cgs.units
Water (85c.c.) bleaching powder (4.7g.)	20	8.0
Water (5X85cc.) bleaching powder (5X4.5g.)	20	10.0
Water (85c.c.) bleaching powder (9.4g.)	12	9.1
Water (5X85c.c.) bleaching powder (5X9.4g.)	12	12.5

A slight rise in fluidity was obtained by the use of a more concentrated bleaching solution, and a more decided rise when five times the volume of the bleaching solution was used for the same weight of grass.

These results show that little degradation results from the bleaching of esparto pulp, provided that the temperature is kept below 45°C and that excessive quantities of a concentrated bleaching solution are not used.

(4) Variation in Fluidity with Time of Digestion.

Esparto grass (100g.) was digested with water (300c.c.) containing sodium hydroxide (16.7g.) under 40 lbs/sq.inch pressure, for different lengths of time. The resulting pulps were washed with running water until free from alkali and bleached for 5 hours with a solution of bleaching powder (bleaching powder (4.7g.) in water (85c.c.)), the proportion of the volume of the bleaching solution to the weight of grass being 85c.c. for every 100g. The resulting bleached pulps were then washed until free from all traces of bleaching solution and dried with alcohol and ether and the fluidities determined. After 1 hour 7.43c.g.s. units, 4 hours 8.05, 8 hours 8.83, 22 hours 9.51 and 44 hours 9.30c.g.s. units.

A small but steady increase in fluidity was observed with increasing time of extraction, up to a period of 44 hours.

(5) /

(5) Variation in Fluidity with Strength of Alkali and Time of Digestion.

Esparto grass (100g.) was digested as in (1) for 13 hours with sodium hydroxide solution (water (300c.c.) sodium hydroxide (8.4g.)) - that is, with a concentration of half that normally used. A little of this pulp was removed from the liquor, washed free of alkali, bleached as in (4), washed free of bleaching solution and dried with alcohol and ether. The remainder of the pulp was digested with the alkaline liquor for a further 23 hours, then washed, bleached as in (4) and dried with alcohol and ether. The fluidity of both these samples was then determined - see Table II.

A further quantity of esparto grass (100g.) was then digested with the usual strength of sodium hydroxide solution (water (300c.c.) sodium hydroxide (16.7g.)) as in (1) for 22 hours. A little of the resulting pulp was removed, washed free of alkali, bleached as in (4), washed free of bleaching solution and dried with alcohol and ether. The remainder of the pulp was digested with the sodium hydroxide for a further 22 hours. The fluidity of both these samples was determined - see Table II.

Esparto grass (100g.) was then digested as in (1) with a sodium hydroxide solution of double the usual concentration - water (300c.c.) sodium hydroxide (33.4g.) - for 4 hours. A small portion of the resulting pulp was removed, washed free of alkali and bleached /

bleached and dried as in (4), while the main portion was redigested in the alkaline liquor for a further 18 hours. A small part of the pulp which resulted was removed, washed, bleached and dried as in (4) and the remainder replaced in the same liquor and digested for a further 22 hours. This resulting pulp was washed free of alkali, bleached as in (4), washed free of traces of bleaching solution and dried with alcohol and ether. The fluidity of all three pulps was determined - see Table II.

Table II

Strength of alkali used. (for every 100g of grass)	Time of Digestion (in hours)	Fluidity (in c.g.s. units)
NaOH (8.4g.) Water (300c.c.)	13	8.56
NaOH (8.4g.) Water (300c.c.)	36	9.96
NaOH (16.7g.) Water (300c.c.)	22	9.51
NaOH (16.7g) Water (300c.c.)	44	9.27
NaOH (33.4g.) Water (300c.c.)	4	8.02
NaOH (33.4g.) Water (300c.c.)	22	8.60
NaOH (33.4g.) Water (300c.c.)	44	16.90

The /

The only significant increase in fluidity, and therefore in degradation, which took place was after 44 hours digestion with a strong solution of sodium hydroxide.

(6) Variation in Fluidity with Time of Digestion with 12% Sodium Hydroxide Solution.

Esparto grass (100g.) was first freed from lignin etc., by a preliminary extraction with a dilute sodium hydroxide solution (water (300c.c.), sodium hydroxide (8.4g.)), for 17 hours as in (1). The resulting pulp, which still partially retained its grass-like structure, was bleached and dried as in (4). This bleached pulp (10g.) was extracted with 12% sodium hydroxide solution (100c.c.) for 6 hours as in (1), the pulp separated from the liquor, washed free from alkali, dried with alcohol and ether and the fluidity determined. Similar quantities (10g.) of the bleached pulp obtained with weak alkali were then extracted with 12% sodium hydroxide solution (100c.c.) for varied lengths of time, and the fluidity of the resulting pulps determined. After 6 hours extraction the fluidity was found to be 7.65c.g.s. units, after $7\frac{1}{2}$ hours 11.71, after 9 hours 14.3 and after 17 hours 17.98c.g.s. units. The pulp obtained by digestion with weak alkali had a fluidity of 7.60c.g.s. units, so the degradation caused by 6 hours extraction with 12% sodium hydroxide solution was almost negligible. After 6 hours the fluidity was /

was found to increase with the time of extraction.

(7) Variation in Fluidity with Time of Extraction with Cold Alkali.

(a) Esparto grass (50g.) was extracted with sodium hydroxide solution - water (300c.c.), sodium hydroxide (16.7g.) - at room temperature, in a ball-mill for 48 hours. A little of the resulting pulp was removed, washed free from alkali, bleached and dried as in (4), while the remainder was digested, in the ball-mill, for a further 48 hours with fresh alkali (300c.c.) of the same strength. After removing a little of this pulp, to be washed, bleached and dried as in (4), the remainder was replaced in the ball-mill for a further 72 hours with fresh alkali (300c.c.) of the same strength. The resulting pulp was washed free of alkali, bleached and dried as in (4), and the fluidity of all three samples determined - see Table III.

Table III

Time of Extraction (in hours)	Fluidity (in c.g.s. units)
48	12.43
Further 48 with fresh alkali	10.14
Further 72 with fresh alkali	14.19

(b) Esparto grass (200g.) was freed from lignin colouring matter, etc, by digestion for 17 hours under 40 lbs/sq.inch pressure with water (600c.c.) containing /

containing sodium hydroxide (16.7g.). After washing free from alkali the resulting pulp was bleached and dried as in (4). This bleached pulp (52g.) was digested with water (1040c.c.) containing sodium hydroxide (52g.) for 1 hour, at room temperature, with continued shaking. The resulting pulp was separated from the liquor, and while the major portion was again shaken with a fresh solution of 5% sodium hydroxide solution (1L) for another hour, a small quantity was washed free of alkali, and bleached and dried as in (4). After this second shaking the main portion was again shaken for 3 hours with fresh 5% sodium hydroxide solution (1L), while a small portion was washed free of alkali, and bleached and dried as in (4). After this third shaking the pulp which resulted was freed from alkali and bleached and dired as in (4), and the fluidities of all three portions determined - see Table IV.

Table IV

Time of Digestion	Fluidity (in c.g.s. units)
Shaken for 1 hour.	5.65
Shaken for a further hour with fresh alkali.	7.02
Shaken for a further 3 hours with fresh alkali.	5.64

Fluidity of Pulp Extracted from De-waxed Esparto Grass at Room Temperature.

Esparto /

Esparto grass was ground up to a coarse powder in a mill and extracted in a Soxhlet extractor eleven times with benzene and four times with methyl alcohol, to remove fat, wax and most of the chlorophyll. It was then bleached and delignified with sodium chlorite - grass (10g.), water (500c.c.), glacial acetic acid (50c.c.), sodium acetate (2g.) and sodium chlorite (50g.). The solution was heated to 60°C for five minutes then kept at 30°C for 24 hours with occasional shaking. The bleached grass was filtered off on cloth, washed with ice-cold water followed by alcohol and allowed to dry in the air.(3)

To extract the xylan the bleached, de-waxed and de-fatted grass (30g.) was treated with 5% sodium hydroxide solution (400c.c.) in a ball-mill, at room temperature for 3 days. The cellulose was filtered off, washed until free from alkali with running water, followed by alcohol and ether and allowed to dry in the air. The fluidity was determined and found to be 15.91 - not appreciably higher than the fluidity of the cellulose obtained by extraction under pressure.

Summary and Conclusions.

The pulps obtained after extraction in the ball-mill showed a higher fluidity than was usually obtained after extraction under pressure, even although the extractions in the ball-mill were carried out at room temperature. This was possibly due /

due to the mechanical degradation of the fibres themselves by the grinding produced by the balls in the ball-mill. After freeing the esparto grass from lignin, etc, by extraction with dilute alkali under pressure, less degradation was naturally observed by shaking in the cold with 5% sodium hydroxide solution than extracting under pressure with 12% sodium hydroxide solution.

These results show that esparto cellulose can be extracted from esparto grass by digestion with sodium hydroxide solution and bleaching with a solution of bleaching powder, without causing much degradation to take place. Degradation was found to have taken place when an excess of strong sodium hydroxide solution was used for a long period of time, and when an excessive quantity of strong bleaching solution at temperatures above 45° was used.

II - Effect of Conditions of Extraction on Pentosan Content.

The method used for determining pentosan contents was that of Marshall and Norris (4). The pulp containing the pentosan (0.2g.) - or pure xylose (0.1g.) for standardising purposes - was placed in a 500ml. flask together with NaCl (22g.) and 13.15% HCl (100ml). The flask was then immersed in an oil bath just up to the level of the acid inside the flask, and the oil bath raised to a temperature of 180°C and maintained therethroughout the distillation. As soon as the acid inside the flask began to boil, 13.15% HCl was added from a dropping funnel at the rate of 30ml. in 10 minutes, until 360 ml. had distilled over. The furfural in the distillate was precipitated by the addition of thiobarbituric acid - three times the weight of furfural expected - freshly dissolved in 13.15% HCl.

The volume of the solution was made up to 500ml. and allowed to stand 20-24 hours, and the flocculent yellow precipitate obtained filtered through a 1 G 3 sintered glass crucible, washed with distilled water (150ml.) and dried at 100°C to constant weight. It was found that washing the precipitate with alcohol (40ml.) after the distilled water produced no loss in weight, and reduced the time of drying necessary.

Accuracy of the Determination. /

Accuracy of the Determination.

All pentosan determinations were carried out in apparatus with ground-glass joints. No rubber was allowed in the apparatus, as this was supposed to affect the determination (5).

To test the accuracy, determinations were carried out with pure, dry, xylose. The furfural obtained was precipitated both with phloroglucinol reagent and thiobarbituric acid, and the latter found to give more accurate results and a derivative which was more convenient to filter. From the weight of derivative obtained, the weight of anhydroxylose was calculated from the following equations:

1) as phloroglucide $y = 0.783P + 0.00397 \pm 0.00103$

2) as thiobarbiturate $y = 0.6293T + 0.0013 \pm 0.00044$

where y = weight of anhydroxylose
P = weight of phloroglucide
T = weight of thiobarbiturate.

Using pure, dry, xylose, the phloroglucinol method of precipitation was found to give an accuracy of 92.9% compared with 98.5% when thiobarbituric acid was used.

Sensitivity of the Determination.

The minimum quantity of xylose necessary to produce a weighable quantity of derivative was sought. A weak, standard, solution of pure, dry xylose was made up in 13.15% HCl, and determinations carried out with this solution using volumes which contained /

contained 1mg., 2mg. and 4mg. of xylose. In the determination using 1mg. of xylose, when the thiobarbituric acid was added to the distillate containing the furfural, the solution turned its customary yellow colour, but no precipitate was obtained. On repeating the determinations with the same amount of xylose and using phloroglucinol reagent, the solution barely took on the usual green colour, showing the thiobarbituric acid to be the more sensitive reagent.

Using 2mg. of xylose the accuracy was found to be 111% and using 4mg. 100%.

Effect of Glucose on the Determination.

An estimation was carried out with xylose and the same weight of glucose, to ascertain whether the presence of glucose would affect the result. Using phloroglucinol to precipitate the furfural the accuracy was found to be 90.8%, and using thiobarbituric acid 98.3%. This appears to show that the pentosan determination can be carried out with fair confidence in the presence of glucose.

Effect of Arabinose on the Determination.

Xylose and arabinose were mixed in the ratio 20:1 - approximately the ratio considered to be present in xylan, and an estimation carried out using this mixture. The accuracy of the determination was 98.5%.

From the results of these standardising determinations, the method adopted is sensitive to quantities of xylan above 2mg., and accurate to about 98% when quantities of over 4mg. are used. The presence of an equal quantity of glucose or of the amount of arabinose likely to be present in xylan, does not affect the determination.

(1) Pentosan content of Pulps after Extraction by Cold Alkali.

(a) Esparto grass (50g.) was heated with water (500c.c.) under 40 lbs/sq.inch pressure for 4 hours in an endeavour to remove part of the encrusting material. The resulting grass (50g.) was then mixed with water (300c.c.) and NaOH (16.7g.) in a ball-mill which was revolved for 48 hours. At the end of this time a little of the pulp was removed, washed free of alkali, bleached with twice the normal volume of bleaching solution (normal volume water (85c.c.) bleaching powder (4.7g.)/100g. grass) washed and air-dried. Similar samples were obtained after the pulp had been treated for a further 48 hours with the same volume of fresh alkali, and then for a further 72 hours with the same volume of fresh alkali. These three samples were then dried under reduced pressure at 60°C over P_2O, and the pentosan contents determined - see Table V.

Table V /

Table V

Treatment given to Pulp	Pentosan Content
48 hours with water (300 c.c.) and NaOH (16.7g.)	19.6%
Further 48 hours with same volume of fresh alkali	15.8%
Further 72 hours with same volume of fresh alkali	12.8%

This method of extraction removed the xylan very slowly, possibly due to the inability of the alkali to penetrate the outer encrusting materials of the grass.

(b) Esparto grass (200g.) was freed from lignin etc. by heating at 40 lbs/sq.inch pressure with water (600c.c.) containing NaOH (16.7g.). The resulting pulp was washed free of alkali, bleached with bleaching solution (water (170c.c.), bleaching powder (9.4g.)), washed free of the bleaching solution and air-dried. This bleached, air-dried pulp (52g.) was mixed with water (1040c.c.) and NaOH (52g.) and shaken in the cold for 1 hour. A little of the resulting pulp was then removed, washed until free from alkali and air-dried. The remainder of the pulp was shaken for a further hour with the same volume of fresh alkali and a sample obtained, then with the same volume of fresh alkali for /

for a further 3 hours. Samples of all three pulps were dried under reduced pressure at 60°C over P_2O and the pentosan contents determined - see Table VI.

Table VI

Treatment given to Pulp	Pentosan content
Shaken for 1 hour	12.57%
Shaken for further 1 hour with fresh alkali	11.09%
Shaken for further 3 hours with fresh alkali	6.48%

More xylan has been extracted by this method than by the ball-mill method, possibly due to the fact that encrusting lignin, colouring matter, etc. had been previously removed, thus facilitating the penetration of the alkali.

(2) Pentosan content of Pulps after Extraction Under Pressure.

(a) Weak Alkali.

Esparto grass (50g.) was heated with water (150c.c.) containing sodium hydroxide (4.2g.) under 40 lbs/sq.inch pressure for 13 hours. A little of this pulp was removed, washed free of alkali, bleached as in (I (4)), washed until free from traces of the bleaching solution, and dried in the air. The remainder of the pulp was heated for a further 23 hours then washed and bleached as before, and /

and the pentosan contents of both samples determined - after 13 hours 27.5% pentosan and after 36 hours 26.1% pentosan was present.

This treatment has little success in removing the xylan, possibly due to the fact that even after 36 hours the encrusting materials had not been fully removed, and the pulp not not disintegrated into fibres.

(b) Strong Alkali.

(1) Esparto grass (50g.) was treated as above in 2(a) but with a more concentrated sodium hydroxide solution - water (150c.c.), sodium hydroxide (8.4g.). Samples of the resulting pulps were removed after 22 hours and 44 hours, washed, bleached, washed and dried in the air and the pentosan contents determined. After 22 hours the pentosan content was 26.3% and fell to 20.8% after 44 hours. This strength of alkali is that which is used in the Industrial isolation of a pulp suitable for use in paper manufacture, but even this is ineffective in removing more than a small proportion of the xylan.

(2) Esparto grass (50g.) was extracted as above (2(a)) but with a solution of sodium hydroxide containing sodium hydroxide (16.7g.) in water (150c.c.). After removal of samples of the pulp, washing, bleaching and washing and drying in the air, the pentosan /

pentosan contents were found to be 18.9% after 22 hours, and 16.6% after 44 hours.

Although the xylan content has been reduced by this method, it has not been removed completely. In order to obtain an Esparto cellulose free from xylan with this strength of alkali, the grass was first freed from lignin with weak alkali (I (6)), bleached, washed and air-dried. This resulting pulp, which still contained practically all the xylan originally present in the grass, was then treated with sodium hydroxide solution of the same strength as in (2 (b)2) under 40 lbs/sq. inch pressure. After 18 hours a small sample of the resulting pulp was removed, while the remainder was replaced with the same volume of fresh alkali of the same strength. Samples were withdrawn after a further 20 hours and 40 hours, the main bulk of the pulp being re-heated under pressure with the same volume of fresh alkali both times. The three samples of pulp were then washed and dried and the pentosan contents determined. The pulp resulting from the third extraction was found to be free from pentosan, but to have a high ash content - 6.67%.

This /

This treatment was much too long and drastic to adopt as a standard method of obtaining an Esparto Cellulose free from xylan, so milder conditions were sought.

(c) Extraction with 12% Sodium Hydroxide Solution.

Esparto Grass (200g.) was first freed from lignin and other encrusting materials with weak alkali (I (6)) and bleached (I (4)) and portions (10g.) heated with 12% sodium hydroxide solution (100c.c.) under 40 lbs/sq.inch pressure for varying periods, the resulting pulps being washed free of alkali, dried and the pentosan contents determined. The xylan was removed much more quickly by this method, 6 hours reducing it to 4.47%, 7½ hours to 1.24%, and 9 hours removing it completely.

This treatment for 9 hours was then adopted to prepare an Esparto Cellulose free from xylan on the large scale. This resulted in an Esparto Cellulose with a fluidity of 14.3 and ash content of 3.96%.

A pulp containing nearly all the xylan present in the grass was prepared by heating Esparto grass (200g.) with water (600c.c.) containing sodium hydroxide (16.7g.) for 17 hours under 40 lbs/sq.inch pressure. The resulting pulp was then bleached (I (4)), washed and air-dried. The pentosan content was found to be 24.9%, ash content 1.99% and fluidity 7.60 c.g.s. units.

After /

After obtaining these two products, an Esparto Cellulose free of xylan, and an Esparto pulp which still contained 24.9% xylan, attempts were made to isolate a pure xylan from esparto grass. It was desired to compare the behaviour of Esparto Cellulose and Esparto pulp (containing 25% xylan) with that of a standard cotton cellulose, and then to correct the results obtained for Esparto pulp for the presence of xylan, to see if the presence of the xylan modified the properties of the associated cellulose. To do this the behaviour of the xylan had to be determined at the same time as that of the celluloses and pulp, and a pure xylan was desired for this purpose.

III - Extraction of Xylan from Esparto Grass.

In order to obtain xylan as pure as possible, the Esparto grass was heated with weak alkali (I (6)) to remove lignin and encrusting materials, and then bleached (I (4)). The resulting pulp still contained nearly all the xylan which was originally present in the Esparto grass, and when it was heated with alkali to remove the xylan, the liquor precipitated a clean xylan.

Three separate methods of precipitating the xylan from this bleached pulp were attempted, all recommended by different authors.

1) Method of Hampton, Haworth & Hirst. (6)

The bleached pulp (100g.) was heated under 40 lbs/sq.inch pressure with water (300c.c.) containing sodium hydroxide (16.7g.). The liquor was filtered off through a Buchner funnel, and the xylan precipitated from the alkaline liquor with an equal volume of alcohol, as recommended by these authors.

2) Method of Jayme & Sätre. (7)

Bleached esparto pulp (200g.) was heated with 12% sodium hydroxide solution (600c.c.) under 400 lbs/sq.inch pressure. The liquor was filtered off from the cellulose as before (1) and the xylan precipitated by the addition of Fehling's Solution (600c.c.) /

(600c.c.), and the blue, gelatinous precipitate filtered off, and washed free of Fehling's Solution. This precipitate - a copper complex - was dehydrated by grinding up with alcohol four times, suspended in alcohol and decomposed by adding hydrochloric acid until the precipitate was colourless. The xylan thus obtained was washed with alcohol and ether and dried at 15m.m. over P_2O_5 at 60°C.

3) Method of Jayme & Sätre. (7)

Bleached Esparto pulp (10g.) was heated with 12% sodium hydroxide solution (100c.c.) under 40lbs/sq.inch pressure for 12 hours. The xylan was precipitated from the resultant liquor by pouring it slowly, with constant stirring, into alcohol (500c.c.) containing glacial acetic acid (200c.c.). The precipitate of xylan was allowed to settle, the supernatant liquor decanted off and the resultant xylan treated four times with alcohol (100c.c.) and finally ether (100c.c.) and dried at 15m.m. pressure over P_2O_5 at 60°C.

The relative purity of the xylans obtained by these three different methods can be seen from Table VII.

Table VII

Table VII

Time of Digestion of Pulp	Method of Precipitation of Xylan	Ash Content	Pentosan Content
17 hours	(1)	68.4%	20.67%
6 hours	(2)	2.58%	55.6%
7½ hours	(3)	2.74%	85.6%
9 hours	(3)	2.11%	90.3%
12 hours	(3)	7.88%	89.8%

The third method of precipitating the xylan-by pouring the alkaline liquor into alcohol + glacial acetic acid - gave xylans which were of considerably higher pentosan content than the other two methods.

Some of the earlier extractions of xylan gave rise to a polysaccharide with a high ash content (20% in some cases). On analysis this proved to be composed mainly of silica - possibly due to digestion of the glass vessel by the strong sodium hydroxide solution when heated under pressure. To prevent this, later extractions were carried out in a copper vessel with resultant decrease in the ash content to less than 5%. This ash was extremely difficult to remove and was only completely removed by fractional precipitation from 6% sodium hydroxide solution by means of alcohol.

(4) An attempt was made to precipitate a pure xylan from the Esparto grass using a completely different method of extraction. A bleached Esparto pulp was extracted from the Esparto grass by a modification of the method of L.E. Wise (3). Esparto grass was first ground /

ground to a coarse powder and this powdered grass (250g.), enclosed in a muslin bag, was extracted eleven times with benzene (2½L) in a Soxhlet extractor. After extraction with benzene the grass was extracted four times with methyl alcohol (2½L) to remove chlorophyll. This was followed by a bleaching and delignification with sodium chlorite. The de-waxed and de-fatted grass (10g.), glacial acetic acid (50c.c.), water (500c.c.) and sodium chlorite (50g.) were heated to 60°C for 5 minutes, sodium acetate (2g.) added to keep to PH between 4 and 5, and the whole kept in a thermostat at 30°C for 24 hours, with occasional stirring.

After filtering through cloth the bleached, delignified, de-waxed and de-fatted grass was washed with ice-cold water, followed by alcohol and allowed to dry in the air, and was then ready for the extraction of the xylan. To do this the treated grass (30g.) was extracted with 5% sodium hydroxide solution in a ball-mill at room temperature for three days. The pulp which resulted was filtered off through cloth and the xylan precipitated from the liquor by pouring into alcohol (500c.c.) containing glacial acetic acid (100c.c.).

The /

The xylan thus obtained was re-dissolved in 5% sodium hydroxide solution (300c.c.) and re-precipitated by pouring into alcohol (300c.c.) containing glacial acetic acid (60c.c.). This re-dissolving in 5% sodium hydroxide solution and re-precipitatinn was continued until the supernatant liquor was colourless.

Half of the xylan which was obtained by this treatment was washed with alcohol four times and ether once and dried in the air - this was xylan (a). The other half, xylan (b), was re-dissolved in 5% sodium hydroxide solution (100c.c.) and re-precipitated by pouring into Fehling's Solution (100c.c.). The copper complex obtained was decomposed by suspending in alcohol and adding 2N HCl until colourless. The xylan was then washed with alcohol containing hydrochloric acid until all the copper was removed, then washed with alcohol and ether and air-dried.

These two samples of xylan were then dried at 15m.m. pressure over P_2O_5, at 60°C, and their ash and pentosan contents determined. Xylan (a) was found to have an ash content of 0.81% and pentosan content of 82.8%, while xylan (b) had an ash content of 0.97% and pentosan content of 93.0%.

Rotation of Xylan.

The rotations of the various samples of xylan were taken in 6% sodium hydroxide solutions. Many varied results were obtained, ranging from $[\alpha]_D^{20}$ - 46° (C.1.0 in 6% NaOH) /

(C1.0 in 6% NaOH) to $[\alpha]^{20}$ - 106°(C.1.0 in NaOH). Although the xylans showing the low rotation were of low pentosan content - about 60% - this did not entirely account for such a low rotation. These abnormally low rotations are in agreement with the opinion of Jayme and Sätre (7) who came to the conclusion that the rotation of xylans depended on the method of extraction, on the strength of sodium hydroxide solution used, and the amount of arabinose present.

Discussion and Conclusions.

Isolation of a pulp from Esparto grass under conditions as near as possible to those adopted in industrial practice to obtain a material suitable for use in paper-making, results in a material which still contains about two-thirds of the xylan originally present in the grass. The complete removal of this xylan without degrading the cellulose unduly is facilitated if the grass is extracted with weak alkali and the grass-like structure destroyed, with removal of lignin and other encrusting materials. This removal of encrusting materials lays the inner structure of the grass open to attack by the alkali.

If the esparto grass is extracted with alkali in the cold without first removing these encrusting materials, even prolonged treatment removes very little xylan but shaking with cold alkali will remove about two-thirds /

two-thirds of the total xylan if encrusting materials are first removed. Even extraction of the grass under pressure will not remove the xylan unless a sufficient volume of a sufficiently strong solution of alkali is used. Determination of the fluidity of these pulps isolated under different conditions indicates that no serious degradation takes place on isolation by pressure or in the cold, provided that a solution of sodium hydroxide of more than 12% is not used, and that extraction with this under pressure is not carried out for more than about 18 hours.

Determination of the fluidity of the various pulps also indicate that the bleaching of the pulps with a solution of bleaching powder will not cause any appreciable degradation if the volume of the bleaching solution does not exceed 3 x 85 c.c. for every 100 g. of grass, and the strength of the bleaching solution does not exceed about 7%. If the temperature is allowed to rise above 45°C during the bleaching process, degradation is liable to occur.

If these conditions are complied with it is possible to obtain an Esparto Cellulose which has been isolated under fairly mild conditions and whose fluidity is about 14 c.g.s. units, compared with 2.5 c.g.s. units for cotton cellulose isolated under the mildest possible conditions. The esparto cellulose obtained under these conditions is a pure-white product,

strongly /

strongly resembling cotton wool, but with less tensile strength, and, on the whole, being somewhat harder in texture.

When precipitating xylan from the liquor obtained by extracting the esparto pulp with alkali, the product is liable to have a high ash content if the esparto pulp is extracted under pressure. The best way to avoid this is to treat the grass with various reagents to get rid of lignin and other encrusting materials, wax, fat, and colouring matter. If the grass is first ground up to a coarse powder, it facilitates the penetration of the reagents, including the sodium hydroxide, and extraction in the cold with 5% sodium hydroxide solution is found to be adequate to remove all but about 1% of the xylan.

Summary of Part I.

1. The fluidity of the pulp was only increased unduly if an excess of strong (greater than 12%) sodium hydroxide was used in the isolation from the grass.
2. Bleaching does not affect the fluidity if the strength of the solution does not exceed 7%, and the volume 255 c.c./100 g. grass and the temperature is kept below 45°C.
3. Complete removal of pentosan from the cellulose was effected after a minimum of 9 hours at 40lbs/sq.inch pressure with 12% sodium hydroxide solution (100 c.c./10 g. grass).
4. Xylan was isolated together with a considerable amount of ash and other impurities - the purest product was obtained after treating the grass according to L.E. Wise - dewaxing, defatting, bleaching and delignifying followed by extraction in the cold with 5% sodium hydroxide solution.

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PART II

INTRODUCTION

Irvine & Hirst (1) isolated a pulp from esparto grass by digestion with alkali, and on acetylation obtained cellulose Triacetate and Xylan Diacetate. On Methanolysis these two compounds yielded respectively α-+β- Methylglucosides and α- + β- Methylxylosides. This experimental evidence led these authors to the view that the pulp obtained from esparto grass was a mixture of cellulose and xylan. Esparto cellulose has been assumed to be identical with cotton cellulose, but this assumption would only be justified if esparto and cotton celluloses reacted in an identical manner under the same conditions.

Hydrolysis of cellulose to glucose and conversion of the cellulose to cellobiose octa-acetate are useful as indications of the similarity or other-:wise of the two celluloses. Monier-Williams (2) hydrolysed cellulose with concentrated sulphuric acid and converted the cellodextrins thus obtained to glucose, in 90.6% yield, by boiling with acid of strength less than 1%. Barsha & Hibbert (3), among others, obtained cellobiose octa-acetate from cotton cellulose by the use of acetic anhydride and concentrated sulphuric acid.

Haworth /

Haworth, Hirst, Owen, Peat and Averill (4) methylated cotton cellulose in the absence of air and were able to show that when the methylated cellulose was hydrolysed, only 2:3:6 trimethylglucose was obtained. The 2:3:4:6-tetramethylglucose expected from non-reducing end-groups was absent, and this led to the suggestion that the chains of cellulose existed as closed loops. Methylation of esparto cellulose under the same conditions, followed by hydrolysis, should show whether any 2:3:4:6-tetramethyl-:glucose is obtained from end-groups, and the isolation of any dimethylglucoses would indicate that the esparto cellulose had branched chains.

The terms α-, β-, and γ-cellulose were introduced by Cross and Bevan in 1904 and Jentgen in 1911, to distinguish between fractions of cellulose on the basis of resistance to, or solubility in, sodium hydroxide solution of mercerising strength (17.5%). The determination is carried out under specified conditions (5), the portion which remains undissolved being termed α-cellulose, and the two which dissolve β- and γ-celluloses. Of these two latter, the β-cellulose is re-precipitated on acidification, while the γ-cellulose still remains in solution. A comparison of the α-cellulose contents of cotton and esparto cellulose ought to give some indication of the degradation caused by the method of isolating the esparto cellulose.

Investigating /

Investigating the reaction of p-Toluene-sulphonyl chloride with cellulose, Honeyman (6) found it to be a heterogeneous reaction, without any noticeable steric effect. Soluble products were not obtained, but no difficulty was experienced in obtaining a degree of substitution corresponding to two tosyl (=p-toluene sulphonyl) groups for every glucose residue. Honeyman's results showed that the lower the viscosity of the cellulose - i.e. the more degraded the cellulose - the greater the substitution in the secondary hydroxyls, while the presence of a large excess of p-toluene sulphonyl chloride apparently inhibited substitution in the secondary hydroxyls. The primary hydroxyl groups seemed to be less affected by the state of degradation of the cellulose and the quantity of reagent used. It was hoped that a comparison of the rates of tosylation and degree of substitution with cotton and esparto celluloses and the pulp which contains 25% xylan would show up any differences in behaviour between the two celluloses, and also whether the presence of the xylan has any effect on the ease of substitution of the associated cellulose.

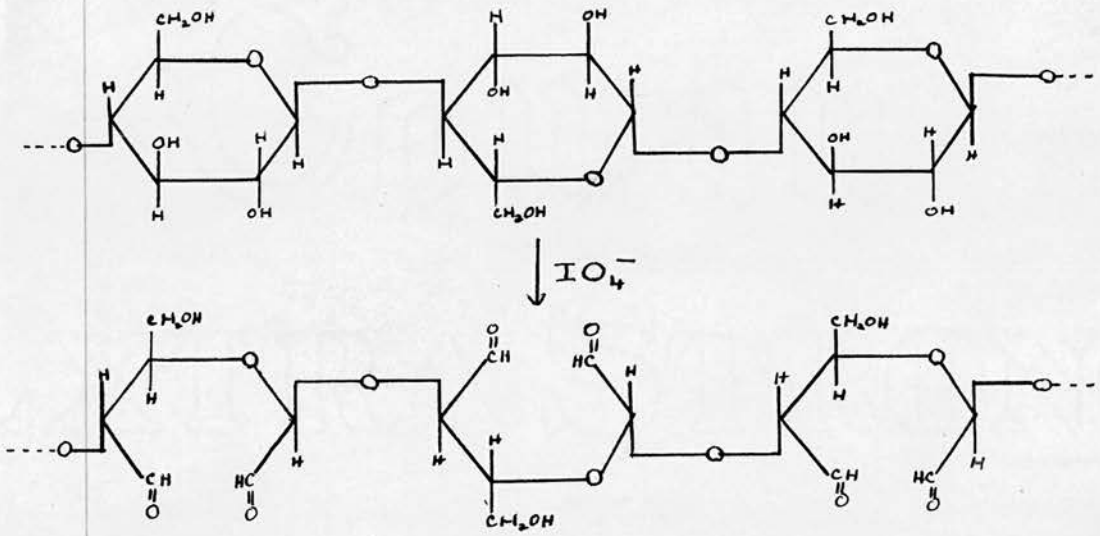
Any compound which contains hydroxyl groups attached to two adjacent carbon atoms can be oxidised by the periodate ion, this oxidation being characterised by a cleavage of the c-c bond, e.g.

$$-CHOH.CHOH- + IO_4^- \longrightarrow -CHO + -CHO + IO_3^- + H_2O$$

The /

The periodate oxidation of an α-glycol of the structure $CH_2OH \cdot CHOH-$ will result in the formation of formaldehyde from the terminal primary alcohol groups.

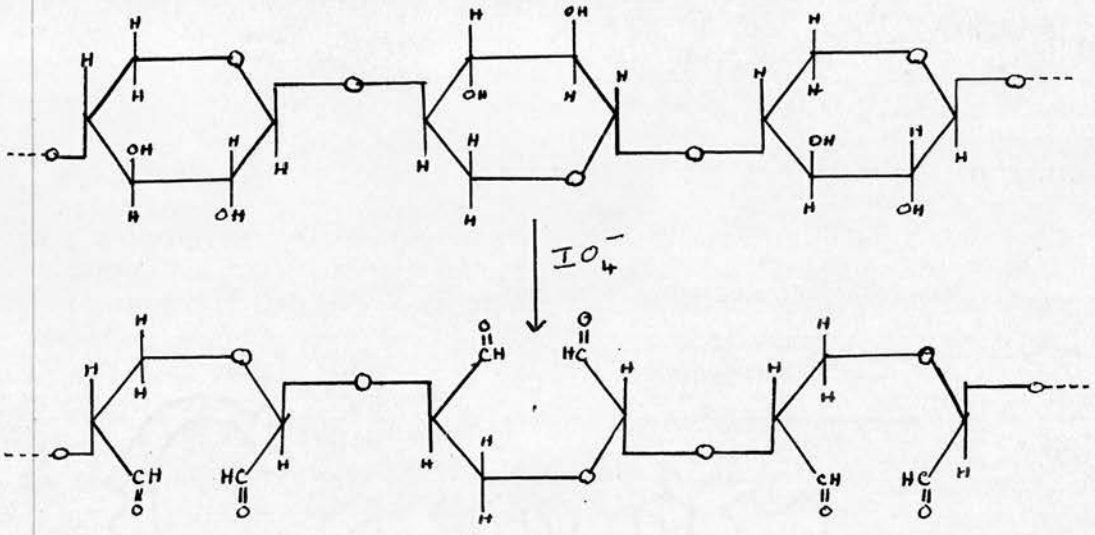
It has already been established [7,8] that cotton cellulose consumes one mole of periodate for every glucose residue present in the chain, giving rise to a polymerised dialdehyde. (See Diagram I).



Polymerised Dialdehyde from Cellulose

Diagram I

According to Jayme and Sätre[9] the oxidation of xylan by the periodate ion pursues a similar course to that of cellulose with the final result that one mole of periodate is consumed for every xylose unit in the chain, giving rise to a polymerised dialdehyde (see diagram II).



Polymerised Dialdehyde from Xylan.

Diagram II

Determination of the fluidity of the esparto cellulose gives an indication of the degradation undergone during the isolation of the cellulose, but does not give any value to the chain-length. Brown, Dunstan, Halsall, Hirst and Jones[10] worked out a method of estimating the chain-length of certain polysaccharides including cellulose by the determination of the formic acid liberated from the end-groups by a solution of potassium periodate. These authors found suitable experimental conditions for the quantitative liberation of formic acid, which was estimated by titration /

titration with 0·1N baryta after destroying the excess periodate with ethylene glycol.

Both reducing and non-reducing end-groups are present in the long, straight chain molecule of cellulose, thus formic acid may be liberated from both the first and the last glucose residue, so three molecules of formic acid may be expected from each cellulose chain (see Diagram III). These authors estimated a value of at least 1,000 glucose residues in the cellulose molecule by this method.

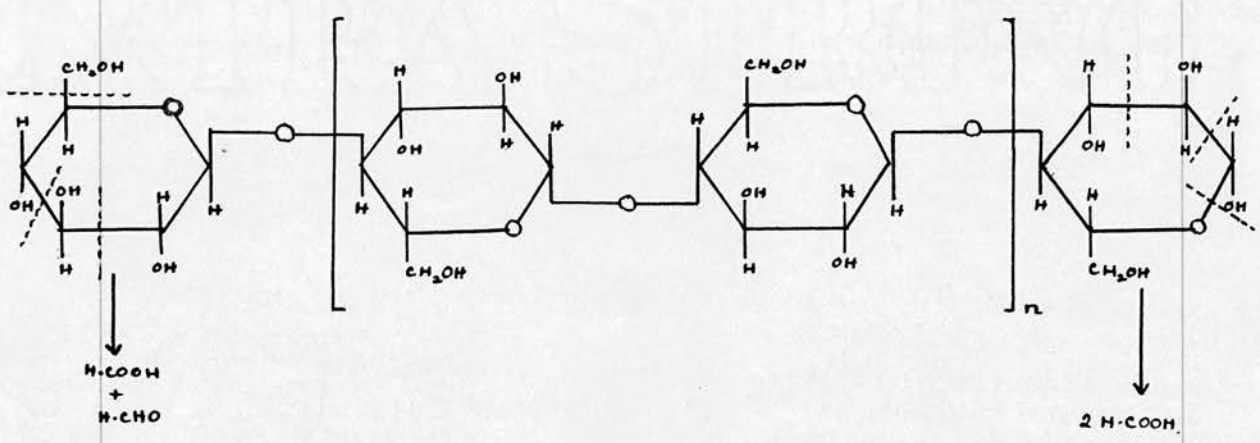


Diagram III

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PART II

EXPERIMENTAL

I Similarity Between Cotton and Esparto Celluloses.

1) Hydrolysis to Glucose.

Cotton and esparto celluloses were hydrolysed to glucose under identical experimental conditions, by the method of Monier-Williams.[1] This entailed leaving the cellulose in contact with 72% H_2SO_4 for seven days, when solution had taken place. The cellodextrins thus formed were hydrolysed to glucose by diluting the acid to less than 1% and refluxing for 17 hours. The glucose present was then estimated by two methods:-

a) Rotation

(1) The syrup from the hydrolysed standard cotton cellulose was found to have a rotation $[\alpha]_D^{20}$ +50·7° (equilibrium value, c. 4·0 in water). As glucose has an equilibrium value $[\alpha]_D^{20}$ +52·5°, 96·5% of the residue was glucose.

The syrup from the hydrolysed esparto cellulose had a rotation $[\alpha]_D^{20}$ +47·1°, (equilibrium value, c. 2·5 in water) corresponding to 93·4% glucose.

b) Somogyi /

b) Somogyi Method.[2]

The Somogyi method of determining glucose and other reducing sugars makes use of the reduction of a solution of copper sulphate. To determine the glucose present in a mixture of reducing sugars, the reducing power of the solution containing the mixture of sugars is found, the glucose removed and the reducing power of the remaining solution measured. The glucose present can be calculated from the difference in the reducing powers of the two solutions.

To remove the glucose from the solution it was incubated with yeast at 38°C for 10 mins. and filtered, the filtrate being a glucose-free solution.[3] Two blanks were needed for every determination - distilled water and distilled water that had been incubated with yeast in a similar manner to the solution containing the sugars.

In the actual determination 5 mls. of the sugar solution, 5 mls. of the same solution freed from glucose, 5 mls. of distilled water and 5 mls. distilled water incubated with yeast were all heated for 10 minutes with 5 mls. of the Somogyi reagent in a boiling water bath. After the reduction of the copper sulphate in the Somogyi reagent had proceeded for /

for 10 minutes the tubes were plunged into cold water to stop the reduction. To each tube was added KI and H_2SO_4 and the liberated iodine titrated with $N/200$ sodium thiosulphate solution. Before the glucose present in the solution can be calculated, the "glucose factor" of the solution has to be determined, i.e., the volume of the $\frac{N}{200}$ sodium thiosulphate which corresponds to 1 mg. of glucose. To do this the same procedure as before is carried out, but with a solution of glucose of known strength - 1 mg./5 ml. solution.

(1) Glucose blank 5·88.

Standard Cotton Cellulose.

Volume of $\frac{N}{200}$ thiosulphate needed to titrate iodine liberated from

a)	Water blank	23·47 ml.
b)	Yeast blank	22·94 ml.
c)	Sugar solution	14·36 ml.
d)	Glucose-free solution	21·92 ml.

Difference due to sugars other than glucose = 22·94-21·92 = 1·02 ml.

Difference due to glucose = 23·47-14·36-1·02 = 8·09 ml.

Glucose present = $\frac{8·09}{5·88}$ = 1·378 mg. (in 5 mls solution)

Glucose /

Glucose obtained from whole solution = 344·5 mg.

$$\% \text{ glucose from cellulose} = \frac{0{\cdot}3445}{0{\cdot}5048} \times 100$$

$$= \underline{68{\cdot}3\%}$$

Esparto Cellulose

Estimated and calculated in the same way, glucose present in the residue after hydrolysis = 0·2790 g.

% glucose from hydrolysis of cellulose = 73·4%

(2) Glucose blank 5·88.

Standard Cotton Cellulose

Estimated as above, glucose present in the residue = 0·4132 g., corresponding to a yield of 79·2% from standard cellulose.

Esparto Cellulose.

Estimated as above, glucose present in the residue = 0·4036 g., corresponding to a yield of 73·7% glucose on hydrolysis.

Comparable yields of glucose are thus obtained on hydrolysis of cotton and esparto celluloses, the low values obtained by the Somogyi method possibly being due to incomplete adsorption of the glucose by the strain of yeast available.

2) Preparation of Cellobiose Octa-Acetate.

The /

The cellobiose octa-acetate was prepared by the method of Barsha and Hibbert.[4] Esparto cellulose was allowed to stand in contact with acetic anhydride and concentrated sulphuric acid at 50°C for 19 days. After dilution with acetic acid, the mixture was poured into water, left overnight and the cellobiose octa-acetate recrystallised from absolute alcohol, (m.p. 227°C).

Esparto cellulose (0·2399 g.), cellobiose octa-acetate (0·0445 g.) m.pt. 224·0°C corresponding to a yield of 18%.

Repetition with esparto cellulose (0·5510 g.) gave rise to cellobiose octa-acetate (0·1928 g.) (m.pt. 224·2°C), corresponding to a yield of 35%.

3) Determination of α-Cellulose.[5]

The determination of the α-cellulose content of a sample of cellulose is a determination of the portion of the cellulose which is resistant to 17·5% sodium hydroxide solution. The cellulose is treated with the sodium hydroxide solution and the α-cellulose washed, dried and weighed according to the method laid down in 1929. The samples of cellulose used are air-dried and thus contain variable moisture contents. The moisture contents of the celluloses used are determined /

determined and the weights of the celluloses used corrected accordingly. Cotton Cellulose (2·7681 g.). α-cellulose present 2.7372 g., corresponding to an α-cellulose content of 98·9%.

Esparto Cellulose (2·7550 g.) α-cellulose (2·5521g) present to 92·6%.

The 6% less α-cellulose present in the esparto cellulose is to be expected from the degradation caused during the isolation from esparto grass.

4) Methylation in Nitrogen.[6]

Esparto cellulose (15 g.) was stirred with 30% sodium hydroxide solution (1500 c.c.) for 4 hours in an atmosphere of nitrogen, then a solution of dimethyl sulphate (150 c.c.) in dioxan (150 c.c.) added over 6 hours and the mixture stirred overnight, with a slow stream of nitrogen bubbled through continuously. The insoluble fibrous product was filtered off through cloth, washed with cold water, squeezed dry by hand pressure, and prepared for a second methylation by soaking in dioxan and kneading under sodium hydroxide solution. Five such methylations were carried out.

After the third methylation the product was washed with hot water and the filtrate heated on the steam-bath /

steam-bath, whereupon the most fully methylated portion separated out. The product after five methylations was washed with hot water, soaked in hot dilute sodium hydroxide solution to remove dimethyl sulphate, washed and dried in vacuo. Yield 8·4 g.

The methylated cellulose (8·4 g.) was dissolved in boiling chloroform (300 c.c.) and ether (50 c.c.), the solution filtered through cotton wool to remove the undissolved, gelatinous portion and the chloroform solution evaporated to dryness in vacuo. The residue obtained was dried at 15 mm. pressure over P_2O_5 at 60°. Yield 2·3 g. (found; OMe 43·9%). This methylated product was purified by precipitation, in granular form, from chloroform solution by light petroleum. (Found; OMe 44·1%).

Hydrolysis of Methylated Esparto Cellulose.

(1) The methylated esparto cellulose (0·1 g.) was hydrolysed by the use of a mixture of HCl (1 c.c.) and acetic acid (glacial) (1 c.c.) which has been shown by Bell[7] to be efficacious in the hydrolysis of resistant polysaccharides. After neutralisation, filtration and evaporation under reduced pressure the residue was extracted with chloroform, taken to dryness /

dryness and chromatographed on paper, (for method, see appendix, p.126). Spots were obtained on the chromatogram with an R_G value of 0·73 (2:3:6-trimethyl-glucose had an R_G value of 0·75 on the same chromatogram). No sugar was present with an R_G value of 1·00 (tetramethylglucose).

(2) The hydrolysis was repeated as above with 1·5 g. methylated esparto cellulose. The residue obtained after the chloroform extraction was converted to the glucosides by boiling with 2% methanolic HCl (50 c.c.) for 18 hours, the residue dissolved in water (40 mls.) and extracted continuously with light petroleum ether (40-60°) in an all-glass 'Quickfit' extractor.[8]

(a) After 3 hours, concentration gave a syrup which was hydrolysed with 0·7 N H_2SO_4 (2 ml.) for 3 hours at 100°C. This residue (0·0727 g.) was dissolved in water and chromatographed. A methylated sugar was obtained with an R_G value of 0·88-2:3:6-trimethylglucose was found to have an R_G value of 0·88 on the same chromatogram. No methylated sugar with an R_G value of 1.00 (2:3:4:6-tetramethylglucose) was present.

(b) 2 days. Concentration gave a syrup (0 4632 g.). A portion (0·0502 g.) was hydrolysed with 0·7 N H_2SO_4 (2 mls.) /

(2 mls) at 100°C for 3 hours and the residue thus obtained chromatographed. A methylated sugar with an R_G value of 0·86 was found to be present. On the same chromatogram 2:3:6-trimethylglucose had an R_G value of 0·86.

(c) 5 days. Concentration of the petroleum ether gave a syrup (0·3312 g.). A portion of this (0·0518 g.) was hydrolysed with 0·7N H_2SO_4 (2 mls.) for 3 hours at 100°C and the residue chromatographed. The chromatogram showed the presence of a methylated sugar with an R_G value of 0·84- 2:3:6-trimethylglucose had an R_G value of 0·84 on the same chromatogram.

The aqueous solution of methylated sugars (40 c.c.) was then concentrated to 10 c.c. under reduced pressure and extracted in an all-glass 'Quickfit' extractor with chloroform for 40 hours.

(d) Concentration of the chloroform gave rise to a syrup (0·2938 g.), of which a portion (0·0508 g.) was hydrolysed with 0·7N H_2SO_4 at 100°C for 3 hours. The residue was chromatographed and was found to contain a methylated sugar with an R_G value of 0·83. 2:3:6-trimethylglucose was found to have an R_G value of 0·83 on the same chromatogram.

(e) /

(e) The aqueous solution was taken to dryness under reduced pressure, giving rise to a white, crystalline residue (0·6527 g.). A portion of this (0·0748 g.) was hydrolysed with 0·7N H_2SO_4 (3 mls.) at 100°C for 3 hours and the residue chromatographed. The chromatogram showed the absence of any sugar or methylated sugar in the crystalline residue.

(5) Determination of Chain-length by Estimation of Formic acid Produced from End-groups by Oxidation with Potassium Periodate solution.[9]

Polysaccharides like cellulose and xylan, built up with chains of hexopyranose and pentopyranose residues linked 1:4 should yield formic acid only from the reducing residues (if any) and the terminal residues.

The oxidation was carried out in a brown bottle (250 c.c. capacity) fitted with a ground-in glass stopper and cleaned by being steamed out. The cellulose or xylan used (0·5 g.), 0·2M $NaIO_4$ solution (25 ml.), distilled water (100 ml.) and KCl (5 g.) were all shaken together. Potassium periodate was precipitated and left a saturated solution of potassium periodate which was sufficient to effect the quantitative /

quantitative liberation of formic acid from the terminal residues of the polysaccharide.

The cellulose or xylan was shaken continuously with the saturated potassium periodate solution for an hour, 10 mls. of the solution withdrawn, excess periodate destroyed by ethylene glycol, methyl red added and the solution titrated with 0·01N NaOH using a micro-burette. This titration acted as a blank, to correct for the solutions not being completely neutral. The polysaccharide and potassium periodate solution were then shaken continuously - 10 mls. were withdrawn periodically and titrated as above until oxidation appeared to have ceased.

Both cotton and esparto celluloses were oxidised under these conditions and their chain lengths calculated. After 14 days, from cotton cellulose (0·9275 g.) formic acid had been liberated corresponding to 1·414 ml 0·013N NaOH - i.e. a chain length of 935. After 17 days, formic acid produced = 1·444 ml - i.e. a chain length of ¶15 anhydroglucose units. After 14 days, from esparto cellulose (0·4620 g.) formic acid liberated corresponded to 0·555 ml. 0·013N NaOH - chain length of 1186, and after 21 days 0·655 ml. - chain length of 1005 anhydroglucose units.

(6) /

(6) Determination of Uronic Acid.

Uronic acid was determined by the method of McCready, Swenson and McClay[10] by the distillation of the polysaccharide with 19% HCl. The carbon dioxide evolved from any uronic acid present was absorbed with 0·25N NaOH and the excess alkali determined by back-titration with 0·1N HCl. No uronic acid was found to be present in the cotton cellulose, esparto cellulose, esparto pulp containing 25% associated xylan, or in xylan itself.

II Study of the Association Between Xylan and Cellulose in Esparto Holocellulose.

1) Tosylation.[11]

In order to study the association between the xylan and the cellulose in esparto holocellulose, the reactivity of the hydroxyls on carbon atoms 2,3, and 6 was compared in cotton cellulose, esparto cellulose, esparto pulp (25% xylan), and the hydroxyl groups in xylan. On treatment with p-toluene sulphonyl chloride the degree of tosylation obtained was found to vary with the experimental conditions - time, temperature, and the state of the cellulose used. The proportion of esterification in the primary hydroxyl group can be estimated by treating the tosylated compound with sodium iodide, when the tosyl group which has substituted the primary hydroxyl group will be replaced by iodine.[12]

The cellulose, pulp or xylan to be used was first dried at 15 mm. pressure at 60°C over P_2O_5 for 3 hours. The polysaccharide thus dried (3 g.) was then treated with p-toluene sulphonyl chloride (9 g.) in dry, re-distilled pyridine (60 c.c.). After various trials it was found that a temperature of 100°C was convenient. After substitution the products were washed /

washed with pyridine, freed from pyridine and dried as before, and the sulphur content determined.

Determination of Sulphur Content.

The tosylated polysaccharide (0·3 g.) was mixed with dried sodium carbonate (2 g.) and sodium peroxide (5 g.) in a closed steel bomb, heated gently and then ignited for 30 minutes. The contents of the bomb were heated gently with water to dissolve sodium salts, and the ferric hydroxide filtered off. The solution was then neutralised with concentrated hydrochloric acid and 1 ml. excess added and after dilution the sulphate precipitated with 10% barium chloride solution. Picric acid was added to increase particle size and allow of immediate filtration.

Substitution with Sodium Iodide.[13]

The tosylated polysaccharide (1 g.), sodium iodide (2 g.) and dry acetone (30 c.c.) were heated to 100°C in a boiling water bath in a glass bottle fitted with screw top and rubber washer, for 2 hours. After the bottle and contents had cooled and the pressure returned to atmospheric the iodinated product was washed with alcohol, followed by N/10 sodium thiosulphate solution to remove adsorbed iodine, washed with water, dried as before and the iodine content /

content determined.

Determination of Iodine Content.

The dried iodinated product (0·2 - 0·25 g.) was refluxed with sodium and absolute alcohol, previously distilled over sodium to remove aldehydes. After diluting and neutralising the solution, 0·1N silver nitrate solution (25 ml.), standardised by Volhard's method, was added, the solution filtered and the filtrate titrated with 0·1N potassium thiocyanate solution, using an iron alum indicator.

Cotton cellulose, esparto cellulose, esparto pulp containing 25% xylan, and xylan were all tosylated and iodinated. In the pulp containing the xylan, the proportion of hydroxyl groups substituted in the cellulose portion (75%) was calculated, subtracting from the total sulphur and iodine contents that amount substituted in the xylan. The sulphur and iodine substituted in the xylan (25%) were calculated assuming that the same amount of substitution would take place when the xylan was associated with the cellulose as it would when alone. For the results of substitution of primary and secondary hydroxyls see table VIII.

TABLE VIII

Material Used	Time of Tosylation (in hrs.) at 100°C.	Primary Hydroxyl Groups substituted per glucose (or xylose) residue.	Secondary Hydroxyl Groups substituted per glucose (or xylose) residue.	Total Hydroxyl Groups substituted per Glucose (or xylose) residue.
Cotton	6	0·11	0·12	0·23
Cellulose	12	0·17	0·07	0·24
fluidity 2·5 c.g.s.units.	24	0·17	0·08	0·25
Esparto	6	0·25	0·06	0·31
Cellulose	12	0·28	0·15	0·43
fluidity 14·3 c.g.s. units.	24	0·36	0·09	0·45
Cellulose in Esparto pulp	6	0·13	0·10	0·23
(Cellulose 75%, Xylan 25%).	12	0·13	0·12	0·25
fluidity 7·6 c.g.s. units.	24	0·15	0·13	0·28
Xylan	6	0·17	0·83	1·0
(90·3% pentosan).	12	0·20	0·80	1·0

The values quoted for the esparto pulp are the values for the cellulose when corrected for the presence of /

of the xylan (25%).

From the results tabulated in Table VIII, it would appear that the cotton cellulose and the esparto cellulose when associated with xylan behave in a comparable manner. The esparto cellulose itself would appear to be more reactive, as there is a marked increase in the proportion of primary hydroxyls esterified. A conclusion which could be drawn is that the primary hydroxyl groups in esparto cellulose are more reactive than those in cotton cellulose, the lower proportion found in the esparto cellulose when associated with the xylan being due to a certain amount of hindrance by the xylan.

Another possibility is that the treatment with 12% sodium hydroxide solution under pressure, undergone by the esparto cellulose during isolation has made more primary alcohol residues available. This appeared a possibility in view of the greater degree of substitution obtained by Honeyman, when using low viscosity (i.e. highly degraded) cellulose and regenerated cellulose.

In order to ascertain whether the difference in substitution of the primary hydroxyl groups was due to increased degradation, cotton cellulose was digested with /

with 12% sodium hydroxide solution under 40 lbs/sq. inch pressure for varying periods. The fluidity of the resulting slightly degraded celluloses was determined, and these celluloses tosylated and iodinated as before - see Table IX.

TABLE IX

Fluidity in c.g.s. units.	Time of Tosylation	Total OH groups.	Primary OH groups.	Secondary OH groups.
2·46	12	0·24	0·17	0·07
7·7	6	0·21	0·13	0·08
16·3	12	0·29	0·19	0·10
23·3	12	0·29	0·22	0·07

The cotton cellulose with a fluidity of 16·3 c.g.s. units was comparable with the esparto cellulose, which had a fluidity of 14·3 c.g.s. units. Although an increase in fluidity would appear to produce a slight increase in substitution, especially of the primary alcohol group, the degree of substitution still does not approach that of the esparto cellulose. It therefore seems reasonable to suppose that when the esparto cellulose is associated with xylan some of the primary /

primary alcohol residues are prevented by the xylan from entering into reaction with p-toluene sulphonyl chloride, and that when the xylan is removed, they are free to react.

Information on the possible hindrance of the reaction of the secondary hydroxyl groups by the xylan was sought by the oxidation of cotton cellulose, esparto cellulose, esparto pulp (25% xylan) and xylan with sodium periodate solution and a comparison of the rates of oxidation and consumption of oxidant.

2) Oxidation by Sodium Periodate Solution.

A 0·20M to 0·26 Molar solution of sodium periodate was used as oxidant, the soluble metaperiodate being obtained from the insoluble paraperiodate by recrystallisation from nitric acid.

The oxidations were carried out in brown bottles (100 c.c. capacity) with ground in stoppers and cleaned by chromic acid followed by running water. The polysaccharides used were air-dried and not dried over P_2O_5, in case this intensive drying should affect the uptake of oxidant. The moisture contents of all samples used were determined, and the weights accordingly corrected. The polysaccharide (0·25 g.) and /

and the sodium periodate solution (25 ml.) were shaken together and kept in a thermostat at 20°C. After one hour, when the materials had come to equilibrium, the quantity of sodium periodate present in the solution was determined, as a blank.

At regular intervals the sodium periodate still present in the solution was estimated, until it ceased to be consumed by the polysaccharide.

The periodate still present in the solution was estimated by weighing out approximately 0·25 g. - having determined the relative density of the sodium periodate solution, the volume could be calculated from this, more accurately than 0·25 ml. could be measured. To the sodium periodate solution was added 20% potassium iodide solution (2 c.c.), excess sodium bicarbonate, and 0·1N sodium arsenite solution (5 ml.). After standing 10-15 minutes to allow the periodate to be decomposed to iodate by the arsenite, the arsenite still present in the solution was determined by titration with 0·1N iodine. These estimations were carried out in duplicate, small quantities being used so that the total decrease in the volume of the solution might be as small as possible.

The sodium periodate solution oxidised adjacent hydroxyl /

hydroxyl groups to the corresponding dialdehyde, and also the end-groups to give formic acid and formaldehyde. Estimations of the formic acid produced indicated that the uptake of periodate by the end-groups of cotton and esparto cellulose was negligible compared with the total uptake. Xylan has a much smaller chain length and the uptake of periodate by end-groups becomes appreciable.

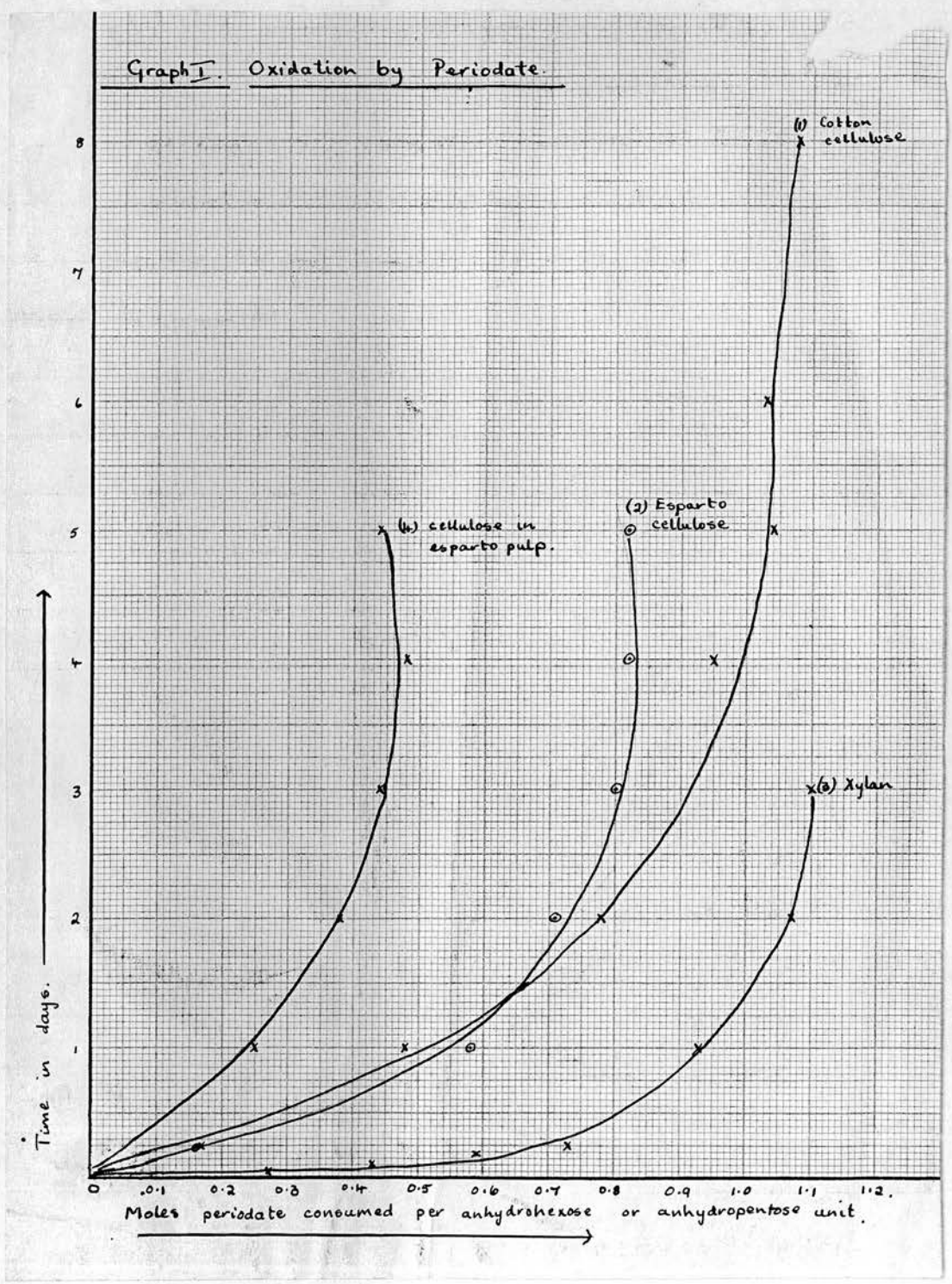
Cotton cellulose, esparto cellulose, esparto pulp (25% xylan) and xylan were all oxidised under similar conditions and the results can be seen below:-

Cotton Cellulose (fluidity 2·5)

0·4698 g. After 6½ hrs., periodate consumed by the cellulose was 0·17 mole, 24 hrs., 0·57 mole, 2 days, 0·83 mole, 3 days 0·80 mole, 5 days 1·15 mole, 6 days 1·08 mole, 7 days 1·23 mole, 8 days 1·22 mole, 9 days 1·11 mole. i.e. the cotton cellulose has consumed slightly more than one mole of periodate for every anhydroglucose unit in the chain.

The oxidation was repeated using 0·5027 g. of the cotton cellulose, with the following results:-

5½ hrs. 0·11 mole, 24 hrs. 0·38 mole, 2 days 0·72 mole, 3 days 0·69 mole, 4 days 0·95 mole, 5 days 0·93 mole, 6 days 0·97 mole, 8 days 0·93 mole. i.e. the cellulose has used slightly less than one mole of periodate per anhydroglucose /


Graph I. Oxidation by Periodate.
(1) Cotton cellulose
(2) Esparto cellulose
(4) cellulose in esparto pulp.
(3) Xylan
Time in days.
8
7
6
5
4
3
2
1
0
.0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2.
Moles periodate consumed per anhydrohexose or anhydropentose unit.

anhydroglucose unit. Graph I (1) represents the average of these results.

In order to determine whether the state of degradation liable to be present in the esparto cellulose would affect the uptake of oxidant, a similar oxidation was carried out with cotton cellulose with a fluidity of 16·3 c.g.s. units.
5 hrs. 0·11 mole, 24 hrs. 0·45 mole, 2 days 0·74 mole, 3 days 0·86 mole, 4 days 0·95 mole, 5 days 1·07 mole, 7 days 1·10 mole, 8 days 1·12 mole. From these results it can be seen that cotton cellulose with a fluidity of 16·3 c.g.s. units behaves in a similar manner to that with a fluidity of 2·5 c.g.s. units.

Esparto Cellulose

Oxidations were carried out under the same conditions with esparto cellulose:-
(1) 0·5086 g. 5 hrs. 0·12 mole, 24 hrs. 0·61 mole, 2 days 0·71 mole, 3 days 0·79 mole, 4 days 0·82 mole, 5 days 0·82 mole.
(2) 0·5033 g. 5 hrs. 0·20 mole, 24 hrs. 0·55 mole, 2 days 0·70 mole, 3 days 0·80 mole, 4 days 0·82 mole, 5 days 0·82 mole.

The oxidation of the esparto cellulose ceased in both cases when 0·82 mole of oxidant had been consumed for /

for every anhydroglucose unit in the chain. Graph I (2) represents the average of these results.

(3) 0·9839 g. After 4 hrs. 0·14 mole periodate consumed / anhydroglucose residue, 24 hrs. 0·35 mole, 2 days 0·48 mole, 3 days 0·54 mole, 4 days 0·61 mole, 6 days 0·67 mole, 7 days 0·67 mole, 8 days 0·68 mole, 9 days 0·78 mole, 10 days 0·77 mole, 11 days 0·80 mole, 13 days 0·80 mole, 16 days 0·80 mole.

Even when the oxidation is allowed to proceed for over twice as long as the previous oxidations, the consumption of periodate still remained at 0·8 mole per anhydroglucose unit.

Further confirmation of this uptake of 0·8 mole periodate/anhydroglucose unit was obtained by a similar oxidation, carried out with 3·5561 g. of esparto cellulose, and 175 ml. of 0·25M sodium periodate solution. The estimations of the periodate present were carried out on a larger scale - 3 g. of the solution being weighed out and excess sodium bicarbonate, potassium iodide (2 g.) and 0·1N sodium arsenite solution (25 ml.) added. The excess of sodium arsenite was determined as usual with 0·1N iodine, and after a period of 12 days the periodate consumed had still not risen above 0·809 mole / anhydroglucose unit.

Esparto /

Esparto cellulose was hydrolysed as above (see p.78) and the residue chromatographed. A sugar with an R_G value of 0·082 was obtained - glucose was found to have an R_G value of 0·083 on the same chromatogram. No other sugars were observed to be present, which demonstrates that the inability of the esparto cellulose to consume sodium periodate is not due to the presence of any other sugars.

Xylan

(1) 0·4876 g. After 1 hr. 0·18 mole periodate consumed / anhydropentose unit, after 2 hrs. 0·40 mole, 4½ hrs. 0·53 mole, 6 hrs. 0·73 mole, 24 hrs. 0·91 mole, 2 days 1·05 mole, 3 days 1·05 mole.

(2) 0·5719 g. After 1 hr. 0·36 mole periodate consumed / anhydropentose unit, 2 hrs. 0·45 mole, 4½ hrs. 0·68 mole, 6 hrs. 0·73 mole, 24 hrs. 0·95 mole, 2 days 1·09 mole, 3 days 1·15 mole. Graph I (3) represents the average of these results.

In order to determine how much of the periodate had been used in oxidising the end-groups of the xylan, the xylan was oxidised with potassium periodate as above (see p. 87), and the formic acid liberated from the end-groups estimated by titration with standard sodium hydroxide.

(1) /

(1) 0·5001 g. After 2 days the formic acid produced was found to be equivalent to 2·35 ml. 0·0243N NaOH, 5 days 3·03 ml., 7 days 4·64 ml., 9 days 5·70 ml., 11 days 7·10 ml., 13 days 7·61 ml., 15 days 7·74 ml. (This gives a chain length of 20·3 to the xylan).

(2) 0·9010 g. After 10 days 20·52 ml. 0·013N NaOH were needed to neutralise the formic acid produced, 13 days 21·88 ml., 15 days 22·43 ml. (This gives xylan a chain length of 23·8.).

The average amount of sodium periodate consumed per anhydropentose unit amounted to 1·10 mole. When corrected for the periodate utilised in oxidising the end-groups - correction calculated from the above estimations - the periodate consumed in the oxidation to the dialdehyde was reduced slightly - from 1·10 to 1·04 mole / anhydropentose unit. The oxidation of xylan thus takes a similar course to that of cellulose with the final result of just over 1 mole of periodate consumed for every anhydropentose unit in the xylan chain, but the reaction is much more rapid than that of cellulose.

Esparto Pulp (Cellulose 75%, xylan 25%)

Esparto pulp (containing 25% xylan) was subjected to /

to oxidation in a similar manner with a solution of sodium periodate. The sodium periodate consumed by the pulp corresponded to the periodate consumed by both the 75% cellulose and the 25% xylan present in the pulp. Assuming that the xylan will be oxidised in a similar manner when associated with cellulose as when free, especially since the rate of reaction is much greater than for cellulose, the portion of the total periodate consumed by the 25% xylan can be calculated, and by difference, the periodate used by the cellulose in the pulp. The following results were obtained for the uptake of sodium periodate by the cellulose in esparto pulp.

(1) 0·3450 g. cellulose (0·4601 g. pulp). 4½ hrs. 0·05 mole, 5½ hrs. 0·11 mole, 24 hrs. 0·32 mole, 2 days 0·47 mole, 3 days 0·47 mole, 4 days 0·53 mole, 5 days 0·50 mole. (0·23M $NaIO_4$ used).

(2) 0·4701 g. cellulose (0·5068 g. pulp). 4½ hrs. none consumed, 24 hrs. 0·18 mole, 30 hrs. 0·23 mole, 2 days 0·30 mole, 3 days 0·40 mole, 4 days 0·42 mole, 5 days 0·37 mole, 6 days 0·38 mole, 7 days 0·38 mole. Graph I (4) represents the average of these results. (0·23M $NaIO_4$ used).

(3) 0·7617 g. cellulose (1·0159 g. pulp). 4½ hrs. none consumed, 24 hrs. 0·11 mole, 2 days 0·25 mole, 3 days /

3 days 0·19 mole, 4 days 0·22 mole, 6 days 0·30 mole, 7 days 0·31 mole, 7 days 0·33 mole, 8 days 0·41 mole, 9 days 0·46 mole, 10 days 0·49 mole, 12 days 0·48 mole, 14 days 0·53 mole, 15 days 0·52 mole. (0·17M $NaIO_4$ used, followed by 0·26M).

Assuming that the xylan has been fully oxidised these results, although somewhat variable, indicate that the cellulose present is not oxidised to more than 0·5 mole oxidant consumed for every anhydroglucose unit, even on prolonged oxidation.

Graph I shows the rates at which cotton cellulose, esparto cellulose, xylan and the cellulose of esparto pulp are oxidised by a solution of 0·23 Molar sodium periodate under similar conditions. Xylan is oxidised most rapidly, the esparto cellulose and cellulose in esparto pulp show a similar course, but the cellulose of the pulp stops at 0·5 mole and the esparto cellulose 0·8 mole. Cotton cellulose is oxidised more slowly than any of the other specimens, but with the result that 1 mole oxidant is consumed for every anhydroglucose unit in the chain.

According to the above results, only 0·8 mole of periodate is consumed for every anhydroglucose unit in the esparto cellulose chain and 0·5 mole for every anhydroglucose /

anhydroglucose unit in the chain of the associated cellulose in esparto pulp. It would appear, therefore, as if 20% of the esparto cellulose should still be in the form of anhydroglucose units, after oxidation, not having been converted into the dialdehyde. To endeavour to ascertain whether this was the case, the oxidised products were washed free of periodate and hydrolysed by sulphuric acid in the method already described for cotton and esparto celluloses, and any glucose present estimated by a) rotation of the solution, and b) by Somogyi method. (See p. 79).

Oxidised Cotton Cellulose (1) The residue obtained after hydrolysis of the cotton cellulose oxidised by sodium periodate was found to have a rotation $[\alpha]_D^{20} + 2 \cdot 1^\circ$ (equilibrium value, c. 1·0 in water), corresponding to a glucose content of 1·2%.

(2) After hydrolysis of a second portion of cotton cellulose oxidised by sodium periodate, the solution obtained was optically inactive. By the Somogyi method, the glucose present in residue (1) amounted to 0·28%, and in residue (2) to 0·33%.

When these residues were examined on a paper chromatogram, however, no sugar was present with an R_G value of 0·083 (glucose) and it must be concluded that /

that in cotton cellulose no glucose units have escaped oxidation.

Esparto Cellulose Oxidised by Periodate.

(1) Hydrolysis gave rise to a residue which was estimated to contain 3·3% glucose, by the Somogyi method.

(2) Hydrolysis gave a residue which contained 0·85% glucose, estimated by the Somogyi method.

(3) An estimation of the glucose present in the residue obtained after hydrolysis gave a value of 4·2% (by Somogyi).

When these residues were examined on a paper chromatogram, a sugar was found to be present with an R_G value of 0·084 (glucose R_G = 0·084 on the same chromatogram), which shows that the esparto cellulose has been incompletely oxidised even although the uptake of periodate ceased.

Oxidised Esparto Pulp (cellulose 75%, xylan 25%).

(1) Hydrolysis gave rise to a residue which was estimated to contain 1·1% glucose, by the Somogyi method.

(2) Hydrolysis gave a residue in which, when estimated by the Somogyi method, the glucose amounted to 1·2%.

(3) Hydrolysis gave a residue with 0·91% glucose, estimated /

estimated by the Somogyi method.

By examining these on a paper chromatogram, a sugar was obtained with an R_G value of 0·016 (glucose R_G = 0·079 on the same chromatogram) showing that here also oxidation was incomplete despite the ceasing of periodate uptake.

Owing to there being a possibility of the incomplete oxidation of esparto cellulose being due to inability of the periodate solution to penetrate to all the esparto cellulose, the oxidation was repeated in the presence of a detergent, to facilitate penetration. One part in a thousand of a detergent was added to the oxidation, and a control set up with the same volume of periodate and detergent, to determine whether the detergent affected the periodate solution. Two different detergents were used - "aerosol," and "Teepol-x".

In the presence of "Teepol-x", the periodate consumed after 24 hrs. was 0·46 mole, 14 days 0·77 mole, 15 days 0·75 mole. The periodate in the control solution (0·2526 g.) decreased by 0·008 g. after 15 days, this decrease being insufficient to affect the total uptake within the limits of experimental error.

In the presence of "Aerosol" the decrease in sodium /

sodium periodate in the control solution (0·273 g.) was appreciable - 0·012 g. after 16 days - and sufficient to affect the total uptake of periodate by the esparto cellulose. After correcting the uptake of periodate for the decrease due to the detergent, after 24 hrs. 0·48 mole was consumed, after 14 days 0·84 mole, 15 days 0·87 mole, 17 days 0·85 mole. Thus even in the presence of detergents the uptake of periodate by the esparto cellulose still falls short of the one mole / anhydroglucose residue.

The oxidised product obtained in the presence of "Aerosol" was hydrolysed (0·44 g.) by refluxing 18 hrs. with water (20 mls.) followed by 18 hrs. with 0·1N H_2SO_4 (25 mls.).[14] The residue obtained was chromatographed and a sugar obtained with an R_G value of 0·083, while glucose had an R_G value of 0·084 on the same chromatogram, showing that oxidation was still incomplete even in the presence of a detergent.

In an attempt to obtain a method of oxidising the esparto cellulose so that one mole of oxidant was consumed / anhydroglucose residue, regenerated esparto cellulose was used.

Regeneration of Cellulose.

Cotton cellulose (4 g.) was shaken overnight with cuprammonium /

cuprammonium hydroxide solution (200 c.c.) in an atmosphere of nitrogen. The cellulose was regenerated from this viscous solution by three different methods:-

1) Pouring the solution into 5% aqueous sodium potassium tartrate (400 c.c.) acidified with glacial acetic acid.

2) Pouring into alcohol (300 c.c.) and glacial acetic acid (40 c.c.).

3) Pouring into dilute HCl (400 c.c.).

In each case the copper was removed by soaking in very dilute hydrochloric acid, and after removal of the acid the regenerated cellulose was dried at 15 mm. pressure over P_2O_5 at 60°C.

(1) Fluidity 8·6 c.g.s. units, ash content 0·01%.

(3) Fluidity 7·5 c.g.s. units.

The original cotton cellulose had a fluidity of 2·5 c.g.s. units and zero ash content, thus the regeneration has brought about a slight degradation.

The cellulose regenerated with sodium potassium tartrate was in a more fibrous form than the other two, and this way was chosen as being the most satisfactory method of regeneration and was used to regenerate esparto cellulose from cuprammonium solution in the same way.

Original /

Original esparto cellulose: fluidity 14·3 c.g.s. units, ash 3·96%.

Regenerated esparto cellulose: fluidity 15·8 c.g.s. units, ash 0·86%.

Although a very slight increase in the fluidity indicates a small amount of degradation of the esparto cellulose, the regeneration has purified the esparto cellulose in that the ash content has decreased considerably.

The regenerated cotton cellulose and regenerated esparto cellulose were then oxidised with sodium periodate solution in the usual way.

Regenerated Cotton Cellulose (0·4448 g.). Periodate consumed after 24 hrs. 0·86 mole, 2 days 0·95 mole, 3 days 1·0 mole, 5 days 1·0 mole.

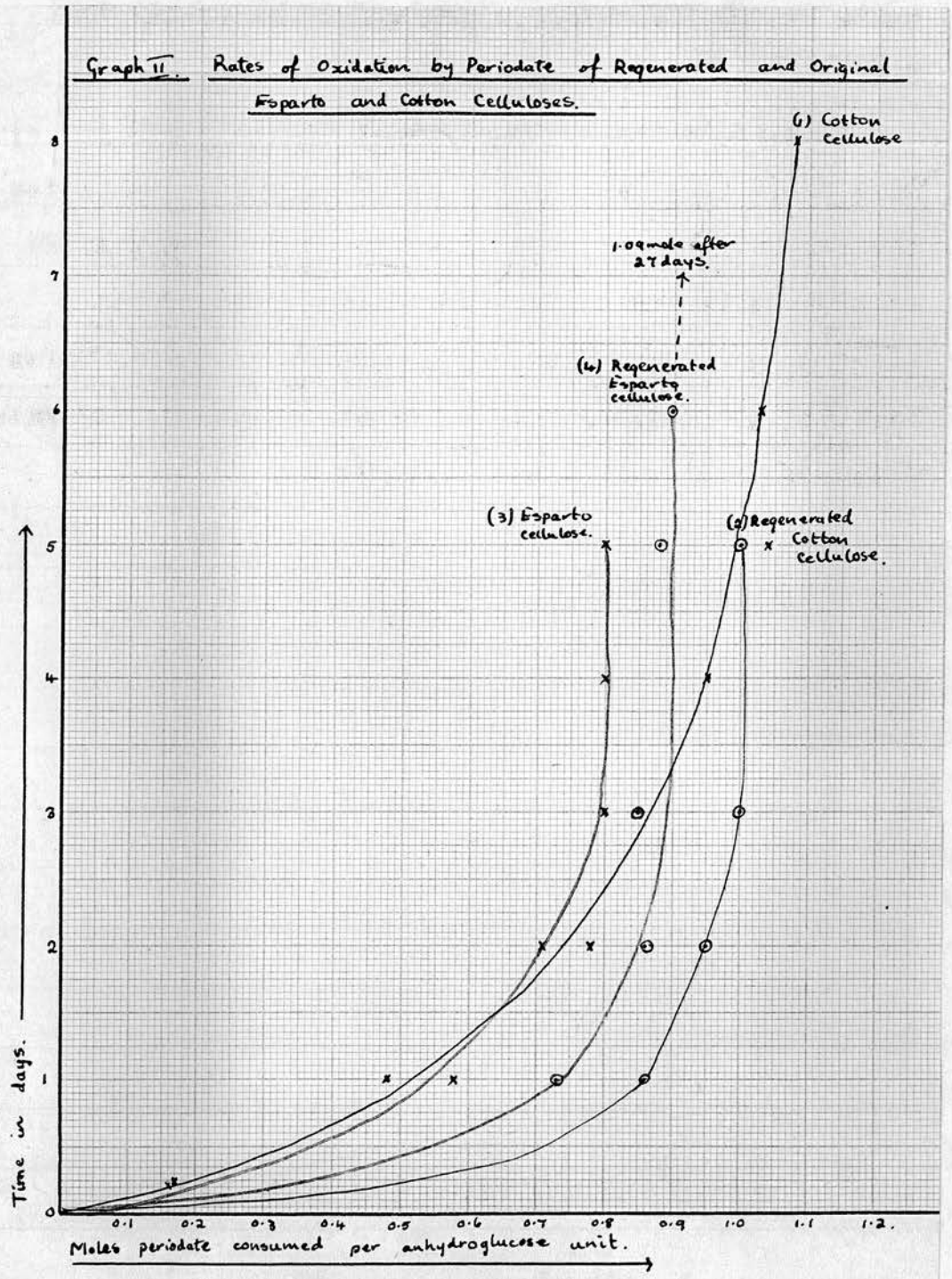
The cellulose has still consumed one mole of periodate for every anhydroglucose unit, but the consumption has been much more rapid than in the original cotton cellulose - see graph II.

Regenerated Esparto Cellulose (0·4410 g.). After 24 hrs. 0·73 mole consumed, 2 days 0·86 mole, 3 days 0·85 mole, 5 days 0·88 mole, 6 days 0·90 mole.

After 6 days the regenerated esparto cellulose consumed 0·9 mole periodate for every anhydroglucose unit /

unit, as compared with 0·8 mole for the original esparto cellulose and 0·85 mole in the presence of "Aerosol."

A second oxidation of the regenerated esparto cellulose (0·4665 g.) was set up and left for 27 days. At the end of this period 1·09 mole of periodate had been consumed for every anhydroglucose unit in the cellulose. This oxidised product (0·3164 g.) was hydrolysed by refluxing with water (12 ml.) followed by N H_2SO_4 (15 mls.) and the residue chromatographed. The chromatograph showed a long "trail," from which it was impossible to identify individual spots. The impurities, probably inorganic salts, were removed by running through ion exchange resin columns. Anions were removed by passing through a column of "Permutit" De-Acidite, and cations by passing through a column of "Permutit" Zeo Karb. H.[15,16] The residue was again chromatographed, and since the "trail" due to impurities was negligible, it was possible to ascertain that no sugar was obtained with an R_G value of 0·083 - i.e. the R_G value of glucose on the same chromatogram. This shows that on regenerating esparto cellulose, the cellulose will consume 1 mole of periodate for every anhydroglucose unit present, while /


Graph II. Rates of Oxidation by Periodate of Regenerated and Original Esparto and Cotton Celluloses.
(1) Cotton cellulose
1.09 mole after 27 days.
(4) Regenerated Esparto cellulose.
(3) Esparto cellulose.
(2) Regenerated Cotton cellulose.
Time in days.
0
1
2
3
4
5
6
7
8
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2.
Moles periodate consumed per anhydroglucose unit.

while before regeneration the oxidation ceases when 0·8 mole has been consumed.

The oxidation of regenerated cotton cellulose is more rapid than that of the original cotton cellulose. The initial rate of the oxidation of the regenerated esparto cellulose is more rapid than that of the original esparto cellulose, but falls off after three days and gradually creeps up to the value of 1·09 mole / anhydroglucose unit - see graph II.

DISCUSSION

It has been shown that, like cotton cellulose, esparto cellulose can be hydrolysed almost quantitatively to glucose. Furthermore, acetolysis gives cellobiose octa-acetate, the highest yield (35%) being comparable with those of 25-35%[17] and 50%[18] reported for cotton cellulose. From these results, therefore, it could not be argued that esparto cellulose and cotton cellulose differ fundamentally.

A further similarity between the two celluloses would appear to be the chain-length - the determination of the chain-length of both celluloses by the estimation of the formic acid produced from the end-groups indicates an average value of approximately 1000 units in the chain in both cases. Determination of the α-cellulose content indicates that slight degradation has taken place, an indication which is borne out by the higher fluidity of the esparto cellulose.

After the methylation of esparto cellulose in nitrogen, subsequent hydrolysis demonstrates the absence of the 2:3:4:6-tetramethylglucose expected if end-groups were present. This is in accordance with the results obtained for cotton cellulose by Haworth et.al., and although carried out on a smaller quantity of /

of methylated esparto cellulose, is obtained by the use of more sensitive methods - i.e. the enrichment of the fractions of the methylated sugars by the extraction method of Brown and Jones,[8] and the separation and identification of the methylated sugars by the use of the paper chromatography technique. The absence of tetramethylglucose, which should be obtained if end-groups were present, points to a structure similar to that postulated by Haworth - the chains of β-D-glucopyranose units existing as closed loops.

2:3:6-Trimethylglucose was the only methylated sugar obtained from the methylated esparto cellulose, which is further evidence of the 1:4 linkages of esparto cellulose. The presence of some 2:4:6-trimethylglucose or 3:4:6-trimethylglucose would have shown that some 1:3 or 1:2 linkages existed as well as the 1:4 linkages in the cellulose, while the absence of any dimethyl-glucoses shows the absence of any cross-linkages.

Any tetramethylglucose which might have existed would have been present in the first fraction obtained in the extraction with light petroleum. Since this fraction amounted to only 0·07 g. it corresponds to a considerable concentration from the original 1·5 g. used /

used and the tetramethylglucose expected from the end-groups should have been in a concentration amply sufficient to have been detected by a paper chromatogram of this first fraction. Similarly, any dimethyl-glucose present would have been extracted by the chloroform used, and since the syrup obtained from the chloroform extract was 0·3 g., this amounted to a considerable enrichment from the original 1·5 g. The resulting concentration would, therefore, have been sufficient to allow of detection on a paper chromatogram. Separation of the trimethyl glucoses is also possible by paper chromatography, since the R_G values of 2:3:6-trimethylglucose and 2:4:6-trimethyl-glucose are 0·82 and 0·77 respectively. Thus the evidence from the methylation of esparto cellulose indicates that esparto and cotton celluloses are chemically similar.

During the methylation of cotton cellulose in nitrogen, Haworth and his co-workers found that although the molecular weight - as determined by the viscosity method - of the cellulose decreased with methylation, no tetramethylglucose was obtained from the end-groups. This was interpreted to mean that although scission of the chains had occurred, the end-groups thus exposed had joined together again, to form smaller loops. Further /

Further work might be attempted on these lines - the methylation of the esparto cellulose repeated and the degradation (if any) during methylation followed by the determination of the viscosity.

The α-celluloses from four different woods were examined by D.J. Bell[19] in an attempt to ascertain whether the celluloses from different natural sources were identical with cotton cellulose. Methylation gave rise to "resistant portions," which could not be methylated to more than OMe = 32% (OMe = 45·6% for trimethyl cellulose) in all four celluloses. Barsha and Hibbert[20] repeated this work using a bigger proportion of methylating reagents and when an apparently maximum methoxyl content had been reached, dissolving the methylated cellulose in chloroform, re-precipitation from which by petroleum ether apparently exposing fresh surfaces and rendering the free hydroxyl groups more readily available to the methylating reagents. These latter authors found none of these celluloses had portions "resistant to methylation" and hydrolysis of these methylated celluloses gave rise to 2:3:6-trimethyl methylglucoside, which indicated behaviour similar to cotton cellulose and furnished evidence as to their chemical identity.

The /

The methylation of esparto cellulose presented no such difficulties and a maximum methoxyl content of 44·1% was obtained after five methylations. The hydrolysis of this methylated cellulose to only 2:3:6-trimethylglucose points to the chemical identity of cotton and esparto celluloses and places esparto cellulose with the four wood celluloses as behaving similarly to cotton cellulose on methylation.

So far the results obtained have gone to show that cotton and esparto celluloses are similar, but differences in behaviour are obtained with various reagents. Substitution with p-toluene sulphonyl chloride, for example, indicates that the primary hydroxyl groups in esparto cellulose are more reactive than those in cotton cellulose. That this increased reactivity cannot be due wholly to the esparto cellulose being more degraded than the cotton cellulose is shown by the fact that cotton cellulose with a fluidity slightly higher than that of the esparto cellulose, and treated in a manner similar to that in which esparto cellulose is isolated, still does not show as much reactivity in its hydroxyl groups as esparto cellulose. When the esparto cellulose is associated with 25% xylan, such reactivity in the primary hydroxyl groups is not shown, which demonstrates that /

that the xylan probably blocks the penetration of the cellulose by the reagents to some extent.

As distinct from an increase in reactivity of the primary hydroxyl groups in esparto cellulose as compared with cotton cellulose, evidence from oxidation with sodium periodate appears to show that the reverse is the case with the secondary hydroxyl groups on C_2 and C_3. Whereas cotton cellulose, as would be expected, consumes one mole of periodate for every anhydroglucose unit, the oxidation of the esparto cellulose stops short when 0·8 mole has been used up for every anhydroglucose residue present. This could mean that the hydroxyl group on either C_2 or C_3 was blocked by the presence of 1:2 or 1:3 linkages in the cellulose, or by the presence of cross-linkages from C_2 or C_3. Both these possibilities are precluded, however, by the evidence resulting from the methylation of the cellulose. If 1:2 or 1:3 linkages had been present, some 3:4:6-trimethyl glucose or 2:4:6-trimethyl glucose should have appeared on the chromatogram, while dimethyl glucose would have demonstrated the presence of cross-linkages. To account for the esparto cellulose only consuming 0·8 mole oxidant / anhydroglucose unit, these latter methylated sugars would have to be present /

present to the extent of 20%, a quantity which could not be missed on a paper chromatogram.

The absence of any other trimethyl glucose apart from the 2:3:6- compound indicates that only C_1 and C_4 are involved in linkage of the units, which furnishes evidence of the absence of other linkages - i.e. 1:2 or 1:3. Cross-linkages between the chains are out of the question because of the absence of any dimethyl glucose and also because any dimethyl cellulose present (glucose) in the methylated cellulose would lower the methoxyl content appreciably from the value to be expected from trimethyl cellulose.

Oxidation of the esparto pulp - cellulose 75%, xylan 25% - showed that when associated with the xylan the cellulose was only oxidised to the extent of approximately 0·5 mole / anhydroglucose unit. This agrees with the evidence furnished by the tosylation of the cellulose, that the xylan blocks the penetration of the cellulose by reagents. The failure of the periodate to oxidise the cellulose fully to the dialdehyde is obviously due here to the two factors - the blockage caused by the presence of the xylan and the inability to penetrate the cellulose noted in the case of the cellulose free from xylan.

Paper /

Paper chromatography has shown that the esparto cellulose is comprised solely of glucose units and since the failure of the esparto cellulose to consume periodate is not due to any chemical difference between esparto and cotton celluloses, the difference between the two is probably due to a difference in their physical structures. This is borne out by the fact that on oxidation of the esparto celluloses in the presence of a detergent, to assist penetration, the consumption of periodate rose slightly from 0·80 to 0·85 mole / anhydroglucose unit - and that on regeneration of the esparto cellulose the uptake of periodate was increased to the expected value of one mole / anhydroglucose unit, although the time of reaction was much greater than for regenerated cotton cellulose.

Various theories have been put forward in the General Introduction as to the nature of the association between the xylan and cellulose. Although the results obtained in this work do not enable a decision to be made between the rival theories of chemical combination or physical association, it seems clear that there is a notable diminution in the reactivity of both the primary and secondary hydroxyl groups of the cellulose when associated with xylan in the esparto holocellulose.

Further /

Further evidence as to the association between the cellulose and xylan could be sought by methylation with methyl iodide, after penetration with thallium ethoxide, of the esparto cellulose when associated with 25% xylan in the esparto pulp. The methylation should be followed by hydrolysis to the methyl glycosides, separation of these by extraction with petroleum ether, by the method of Brown and Jones[8] and paper chromatography of the fractions obtained. This would demonstrate the presence of any methylated sugars apart from the 2:3:6-trimethyl glucose and 2:3-dimethylxylose expected from the cellulose and xylan respectively. The presence of any methylated sugars apart from these two should indicate whether any cross-linkages exist between the cellulose and xylan chains.

Some doubt has been thrown on the existing formula of xylan - i.e. 18-20 D-xylopyranose units terminated at one end of the chain by an L-arabofuranose unit. It has been considered possible that the arabinose might be present as an araban, associated with a xylan composed of a chain of D-xylopyranose units. Paper chromatography of the xylan (a sample not extensively purified) has shown that some arabinose /

arabinose is still present but gives no indication whether it is part of the xylan molecule or merely associated with it.

If the xylan-araban theory proves to be correct, then the esparto holocellulose must consist of at least three components. It is possible that the associated polysaccharides might affect the reactivity of the hydroxyl groups in the cellulose in different ways, e.g. the araban might prevent the oxidation of the secondary hydroxyl groups by periodate while the xylan portion might modify the reactivity of the primary hydroxyl groups, or vice versa, either by purely physical "solvent proofing" effects or by hydrogen bonding. The results of studies on xylan now being carried out in this laboratory may throw some light on this question.

SUMMARY OF PART II

I. Similarities between Esparto and Cotton Celluloses.

1) Hydrolysis to glucose in almost quantitative yield.

2) Acetolysis to cellobiose octa-acetate in comparable yields, providing evidence as to glucose units linked 1:4 as in cellobiose in esparto cellulose.

3) Methylation in nitrogen gave rise to no tetramethyl glucose expected from end-groups and only 2:3:6-trimethyl glucose, evidence of the lack of branching and cross-linkages in esparto cellulose.

4) Comparable α-cellulose contents.

5) Similar chain-lengths - average value of 1000 units.

II. Differences between Esparto and Cotton Celluloses.

1) Substitution with p-toluene sulphonyl chloride reveals that the primary hydroxyls are more reactive in esparto cellulose than in cotton cellulose.

2) Oxidation with from 0·20M to 0·26M sodium periodate indicates the secondary hydroxyls on C_2 and C_3 are less reactive in esparto cellulose than in cotton cellulose. In both these latter cases the xylan is found to hinder the substitution when associated with the cellulose.

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APPENDIX

Filter Paper Chromatography of Sugars.[1]

The solution to be examined is applied as a circular spot near the top of a strip of filter paper. The strip of filter paper is hung with its upper edge hanging in a trough of water-saturated solvent, and the whole kept in a closed vessel in which the atmosphere is kept saturated with water and solvent vapour. A sharp horizontal liquid front forms and advances down the paper at the rate of approximately 35 cms. in 24 hrs., partition of the solute taking place between the water held by the paper and the solvent.

The distance moved by any solute is a function of:

a) The partition coefficient of the solute between water and the wet solvent.

b) The volume of water bound by unit area of the paper.

c) The volume of wet solvent held by unit area of the paper after irrigation.

In any single experiment (b) and (c) will be constant if the filter paper is uniform in texture, thickness and water content, so in ideal conditions the /

the relative distance (R_F) moved by any solute is dependent only on its partition coefficient.

$$R_F = \frac{\text{distance moved by solute}}{\text{distance moved by advancing front of liquid.}}$$

It is the 'R_G' value of a sugar that is usually quoted now and not the 'R_F' value. All sugars are related to 2:3:4:6 tetramethyl glucose, which is given an R_G value of 1·00, i.e.

$$R_G = \frac{\text{distance moved by sugar}}{\text{distance moved by 2:3:4:6-tetramethyl glucose.}}$$

Experimental.

Whatman No.1 filter paper is used, cut into strips approximately 40 cms long and 11 cms wide. A 1% solution of the sugars is the concentration commonly used, and spots are placed on a line 10 cms from the strip and 1·5 cms left between each spot. The solvent used is butanol (40 c.c.), ethanol (10 c.c.) and water (50 c.c.), shaken up together and the butanol-ethanol layer is used in the trough while the water layer is kept in a container inside the closed vessel, to keep the atmosphere saturated.

48 hrs. running was used for sugars, while 18 hrs. was found to be a maximum for methylated sugars. After running for the appropriate time the solvent was removed /

removed by drying at 100°-105°C for ½ hr., the paper sprayed with a mixture of equal parts 0·1N $AgNO_3$ and 5N ammonia solution and developed by replacing in the oven for 10-12 minutes.

Chromatography used to Determine the Constituents of Xylan.

Xylan (0·0511 g.) was hydrolysed with 0·5N H_2SO_4 (5 c.c.) for 3 hrs., while another portion (0·0460 g.) was hydrolysed with 0·2N H_2SO_4 (5 c.c.) for 24 hrs., both at 100°C. After neutralisation and concentration the residues were dissolved in water (1 c.c.) and chromatographed.

In both cases sugars were obtained with R_G values of 0·120 and 0·143, arabinose and xylose were found to have R_G values of 0·121 and 0·144 respectively, on the same chromatogram. Also a trace was obtained in both cases of a sugar with an R_G value of 0·083 - glucose had an R_G value of 0·083 on the same chromatograph.

These results show that both xylose and arabinose were present in the sample of xylan, while glucose was also present as an impurity.

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